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Volume 45 APRIL, 1959 Number 2

THE USE OF PURIFIED ANTIGENS IN THE DIAGNOSIS OF CLONORCHIASIS SINENSIS BY MEANS OF INTRADERMAL AND COMPLEMENT FIXATION TESTS

E. H. SADUN,* B. C. WALTON,* A. A. BUCK,** AND B. K. LEE**

The limited research which has been conducted on the immunodiagnosis of clonorchiasis has yielded equivocal results. Chung and his coworkers used a saline extract of the adult worms in the intradermal and complement fixation tests. Two clonorchiasis and 18 paragonimiasis patients tested with this antigen showed a dermal sensitivity (Chung et al, 1955). The sera from 3 clonorchiasis and 25 paragonimiasis patients gave a positive complement fixation reaction (Chung et al, 1956). The intradermal test with a crude antigen was carried out in 136 infected individuals and 58 negative controls with unsatisfactory results (Hunter et al, 1958). Wykoff (1958) was able to detect antibody response in infected rabbits by the use of an ether extraction of the worms in the complement fixation test. However, even with this antigen, pre-infection rabbit sera reacted to some degree and antibody response had to be evaluated on a relative basis. These few reports based on limited experimentation suggested that further work on this problem was needed, with increased efforts toward the purification of the antigens. Therefore, the experiments reported in this paper were carried out.

MATERIALS AND METHODS

Antigens for the intradermal and complement fixation tests were prepared from adult *C. sinensis* obtained from rabbits experimentally infected 2 months previously with between 800 and 1000 metacercariae each. Metacercariae obtained by pepsin digestion of fish collected in endemic areas in Japan, were washed, counted and forcibly fed to the rabbits. The animals were sacrificed by exsanguination and the intact worms were removed from the biliary passages, washed repeatedly with physiological saline, rinsed with distilled water, transferred to ampoules, rapidly frozen and lyophilized. Four antigens were prepared from the adult worms; a relatively crude fat-free antigen (CC), a lipid-free, borate buffer extraction (CTP), an acid-soluble protein fraction (CM), and an acid-insoluble but alkali-soluble protein fraction (CM-ins). The CC antigen was prepared following essentially the technique described by Chaffee and his coworkers (1954), and the other 3 fractions were prepared according to the method described by Melcher (1943) for the preparation of purified *Trichinella spiralis* antigens. Protein determinations, standardization of the antigens, performance of the complement fixation and of the intradermal tests were carried out as described previously (Sadun et al, 1959a). The control solution for the intradermal tests consisted of buffered physiological saline containing 1:10,000 merthiolate (BSM).

Preliminary tests indicated that all positive intradermal reactions were of the immediate type and reached their peak approximately 15 minutes after injection. At this time, the limits of each wheal were outlined and the areas measured as described previously (Sadun et al, 1959a). The standards for positivity and negativity were the same as those described for the intradermal tests for schistosomiasis (Sadun et al, 1959b). Individuals from endemic areas in Korea and Japan with proven clonorchiasis were subjected to the intradermal test. In addition to *C. sinensis*, most of these patients also harbored various intestinal helminths and protozoa. Individuals from non-endemic areas who were negative for *C. sinensis* by stool examinations

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were used as negative controls. Paragonimiasis patients from Korea and schistosomiasis patients from Japan were selected for a study of the extent of cross reactivity of the intradermal test in these trematode infections.

Sera for the complement fixation test came from individuals with proven clonorchiasis and from trematode-free controls. In order to evaluate the degree of specificity of the reaction, sera from individuals with proven schistosomiasis, paragonimiasis, leprosy, tuberculosis, virus hepatitis and leptospirosis were tested. Rabbit sera were also used to study the development of antibodies in experimentally infected animals. The rabbits were given a single dose of between 800 and 1000 metacercariae, bled just before being fed the metacercariae and at various intervals thereafter. Two other rabbits were inoculated intramuscularly with various amounts of antigenic material (CC and CM-ins) adsorbed onto aluminum hydroxide (Dorin, 1946). Uninfected rabbits were bled from time to time as source of negative control serum.

RESULTS

1. *Intradermal Test.* Preliminary tests designed to compare the reactions produced by 3 fractions of the adult worm antigen (CC, CM, CM-ins) indicated that the CM antigen produced the most reliable and consistent results. Because of these preliminary observations, this antigen was used in the following series together with the saline control (BSM). The results of the intradermal tests in 395 individuals are summarized in Table I. All but 3 of the persons infected with *C. sinensis* (Group I) gave positive reactions whereas all but 2 of the trematode-free con-

TABLE I. *Cutaneous reactions to a purified* C. sinensis *antigen (CM) in persons with clonorchiasis, schistosomiasis, paragonimiasis and in trematode-free controls.*

Group	Trematode infection	Number of persons in group	Number of positives	Percent of positives	Mean size of wheal in mm^2
I	*C. sinensis*	132	129	98	141
II	*S. japonicum*	50	5	10	72
III	*P. westermani*	93	53	57	93
IV	No trematodes	120	2	2	49

trols (Group IV) reacted negatively. Cross reactions occurred particularly with people infected with *P. westermani* (Group III). However, the wheals produced by the homologous antigens were always larger and more distinct, permitting a differentiation when homologous and heterologous antigens were used concurrently.

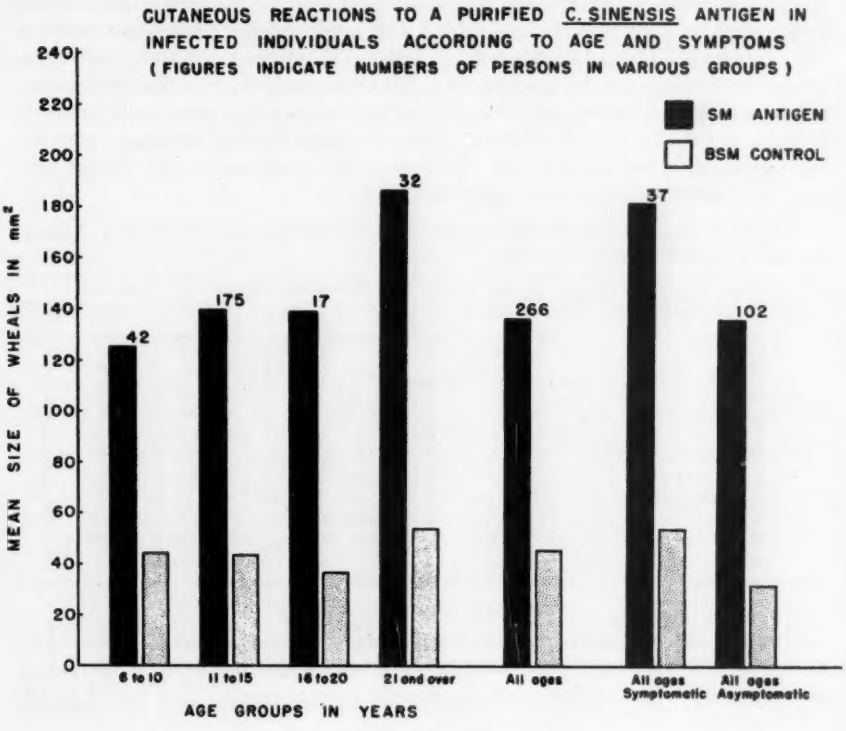
Since most of the infected persons in the above series were adults who had come to the hospital complaining of symptoms directly attributable to clonorchiasis, another experiment was set up to study whether positive specific reactions could be observed in younger, presumably asymptomatic individuals, who would not have been infected repeatedly over a long period of time. A total of 356 children between 8 and 14 years of age from a primary school in a *Clonorchis* endemic area in south Korea were selected for this study. Single stool specimens were examined by the formalin-ether concentration technique (Ritchie, 1948). Determinations of the number of eggs present were done in *Clonorchis* positive specimens. As indicated in Table II, a close correlation between the results of stool examinations and intradermal tests was observed. Discrepant results were obtained in 38 individuals who gave a negative intradermal test but were found to have eggs in the stools and 10 in whom no eggs were found in spite of a positive dermal test. No correlation was observed between the number of eggs in the feces and the size of dermal reactions. An analysis of the degree of the reaction in relation to age, sex and the presence of symptoms was carried out in infected individuals. No significant differences in response between males and females were observed. In both sexes, however, the mean size

TABLE II. *Comparison of results of stool examinations and intradermal test with a purified* C. sinensis *antigen (CM) in a primary school.*

Intra-dermal tests	Results of stool examinations					
	Positive		Negative		Total	
	Number	Percent	Number	Percent	Number	Percent
Positive	148	94	10	6	158	100
Negative	38	19	160	81	198	100
Total	186	52	170	48	356	100

of the wheal appeared to increase somewhat with age (Fig. 1). Also, significantly larger wheals were encountered on the average among individuals who complained of symptoms attributable to liver fluke infection than among the asymptomatic persons.

2. *Complement Fixation Test.* A preliminary experiment was set up to test various antigenic fractions of the adult worms in the complement fixation reaction. Box titrations for each of the 4 antigens were performed using human and rabbit antisera. None of the antigens tested appeared to have marked anticomplementary activity and all of them reacted positively with positive control sera. CM-ins and CTP appeared to be the most sensitive of the antigens tested. The results of testing 181 human serum specimens with CM-ins antigen are summarized in Table III. Ninety-two per cent of the sera from clonorchiasis patients gave positive CF reaction



whereas all but 1 of the sera from uninfected controls gave negative reactions. No false positive reactions were observed among individuals with proven infections of

TABLE III. *Results of complement-fixation test with a purified antigen (CM-ins) in persons with various proven infections and in uninfected controls.*

Diagnosis	Number of specimens	Positive C-F tests	
		Number	Percent
Clonorchiasis	25	23	92
Leprosy	12	0	0
Tuberculosis	58	10	17
Virus hepatitis	12	0	0
Leptospirosis	8	2	25
Paragonimiasis	56	19	34
Schistosomiasis	21	4	19
Uninfected controls	20	1	5

leprosy and virus hepatitis. However, a varied number of individuals from Korea, Japan and the United States with tuberculosis, leptospirosis, paragonimiasis and schistosomiasis gave seemingly false positive results. Since most of these patients lived in areas in which clonorchiasis is present and since no stool samples could be obtained from these individuals, it is impossible to determine at present how many, if any, of these individuals were infected with *C. sinensis* or *Metagonimus yokogawai*, in addition to the specific conditions for which they were under observation and treatment.

The development of antibody response to *C. sinensis* was studied in 9 experimentally infected rabbits and the results obtained with CTP and CM-ins antigens were compared. As indicated in Table IV, 9 pre-infection rabbit sera reacted at low titers with CM-ins antigens but not with CTP antigen. No other differences were observed between the two antigens. Maximal reactivity was reached between 90 and 150 days following infection, after which there was a decrease in antibody levels up to the end of the experiment. Two rabbits artificially immunized with CC and CM-ins gave titers of 1:16 and 1:32 respectively when tested with CM-ins antigen. Pre-inoculation sera were negative.

TABLE IV. *Results of complement fixation tests with CM-ins and CTP antigens in rabbits experimentally infected with* C. sinensis.

Length of infection in days	Number of sera	Antigen used	Number of positives	Complement fixation titer (Reciprocal)							
				2	4	8	16	32	64	128	256
0	9	CM-ins	9	4	4	1	0	0	0	0	0
		CTP	0	0	0	0	0	0	0	0	0
30	2	CM-ins	2	0	1	1	0	0	0	0	0
		CTP	2	0	1	1	0	0	0	0	0
60	7	CM-ins	7	0	0	3	1	0	1	2	0
		CTP	7	0	0	3	1	0	1	2	0
90	5	CM-ins	5	0	0	0	1	0	0	4	0
		CTP	5	0	0	0	1	0	0	4	0
120	4	CM-ins	4	0	0	0	0	1	2	1	0
		CTP	4	0	0	1	0	0	2	1	0
150	4	CM-ins	4	0	0	0	0	2	0	1	1
		CTP	4	0	0	1	0	1	0	1	1
180	3	CM-ins	3	0	1	0	1	1	0	0	0
		CTP	3	0	1	0	1	1	0	0	0
210	2	CM-ins	2	1	1	0	0	0	0	0	0
		CTP	2	1	1	0	0	0	0	0	0

SUMMARY AND CONCLUSIONS

Purified antigens were prepared from adult *Clonorchis sinensis* and tested on known positive and negative individuals. The intradermal test using as antigen an acid-soluble protein fraction gave consistently reliable results. All but 3 of 132

clonorchiasis patients gave positive reactions whereas only 2 of 120 trematode-free individuals were positive. Excellent correlation between results of the intradermal test and stool examinations occurred even when 356 young asymptomatic individuals were tested, although presumably many infections were light and recently acquired. Apparently, by use of this purified antigen, it was possible to eliminate most of the false positives and false negatives reported by Hunter et al (1958). Moderate cross reactions were observed in individuals with paragonimiasis and schistosomiasis. This may not be a serious obstacle in clinical practice since these diseases are, as a rule, clinically distinct from each other and from clonorchiasis. Since the reaction to the homologous antigen is usually much greater, when surveys are conducted in areas where 2 or more of these trematodes are endemic, it should be possible to differentiate infections by introducing the respective antigens at the same time and by comparing the areas of the ensuing wheals.

A sensitive and specific complement fixation test was obtained using as antigens an acid-soluble, alkali-insoluble protein fraction or a lipid-free borate buffer extraction of the adult worms. Two of 25 sera from clonorchiasis patients reacted negatively and only 1 out of 20 sera from trematode-free persons gave a positive reaction. Some cross reactions with individuals infected with paragonimiasis, schistosomiasis, tuberculosis and leptospirosis were observed. Attention should be called to the fact that all of the sera from tuberculosis patients and from leptospirosis which gave false positive results were old. It is possible that some of these seemingly false positive results were due to the storage of sera and/or to the fact that the individuals involved might have had infections with clonorchiasis, or related trematode infections. Promising results were also obtained with sera from rabbits. However, CM-ins reacted at low titers with pre-infection rabbit sera and the antibody response following infection had to be evaluated on a relative basis. No positives with pre-infection sera were observed with CTP antigens.

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RESEARCH NOTE

SWINE AS POTENTIAL RESERVOIR HOSTS OF *HEPATICOLA HEPATICA.*

Lubinsky (1956, Canad. J. Comp. Med. **20**: 457) reviewed the host range of *Hepaticola hepatica,* which is primarily a parasite of rodents and occasionally of man and other animals. Although it has been reported from the peccary by Foster and Johnson (1939, Tr. Roy. Soc. Trop. Med. and Hyg. **32**: 639), insofar as the writer is aware it has not been reported from the domestic pig. Eggs of *H. hepatica,* obtained from the livers of wild rats by peptic digestion, were embryonated according to the method described by Luttermoser (1938, Am. J. Hyg. **27**: 275). These eggs were subsequently fed to two 3-week-old pigs which were necropsied 21 and 30 days later. In both animals, circumscribed, milky-white lesions about 1 to 2 mm in diameter, resembling the "milk-spot" lesions caused by *Ascaris suum* (Fig. 1), were found on the liver surface and scattered throughout the parenchyma. In the animal killed after 21 days only juvenile worms were recovered from the lesions; however, liver lesions of the second animal yielded both juvenile and mature egg-bearing females. Eggs from these females were later embryonated and proved infective when fed to a mouse.

This finding is significant because some lesions formerly considered to be caused by *A. suum* may, in fact, be the result of infection with *H. hepatica;* furthermore, the pig may be involved in the cycle resulting in occasional infections with this parasite in man.—FRANCIS G. TROMBA, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

FIGURE 1. Lesions in liver of pig killed 30 days after infection with *Hepaticola hepatica.* (×2).

STUDIES ON THE FAMILY BUCEPHALIDAE POCHE 1907 (TREMATODA) FROM PHILIPPINE FOOD FISHES

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Trematodes of Philippine food fishes have been little studied, much less the family Bucephalidae. The check list (Tubangui, 1947) of parasitic worms reported from the Philippines shows only 1 adult bucephalid, *Prosorhynchus triangularis* Tubangui and Masilungan, 1944 from the intestine of the freshwater fish *Glossogobius giurus* Buchanan-Hamilton, commonly called *bia* or *biang puti* (Tagalog).

Recent reviews of this group are available (Eckmann, 1932; Verma, 1936, 1936a; Nagaty, 1937; Srivastava, 1938; Manter, 1931, 1940a, 1947, 1954; Chauhan, 1943, 1954; Dollfus, 1951; Kniskern, 1952; and Hopkins, 1954). In view of these reviews a general account of the family will not be presented.

The present paper deals with the family Bucephalidae representing a part of a collection made during a survey of helminthic infection in Philippine food fishes. About 102 fishes representing 11 genera and species yielded 6 genera and 13 species of bucephalids, 12 of which are considered new to science. The fish hosts were bought from Manila markets and vicinity; information on the locality was usually supplied by the market vendors. The methods used were the same as reported by me in previous papers.

All measurements are in millimeters unless otherwise stated; drawings were made with the aid of a camera lucida.

Collection of materials was made possible through a grant-in-aid from the Natural Science Research Center, University of the Philippines. I am greatly indebted to the Guggenheim Memorial Foundation and the University of the Philippines for the Fellowship grant under which the present work was undertaken. I also wish to express my gratitude to Dr. George R. LaRue, Animal Disease and Parasite Research Division, Agricultural Research Center, United States Department of Agriculture, Beltsville, Maryland, for placing at my disposal his library and laboratory; for his very valuable suggestions, criticisms and sympathetic interest in the work; and for critically going over the entire manuscript before it went to press. I also wish to express my thanks to Mr. Allen McIntosh of the same institution for the use of certain reprints and the loan of the type specimens in the collection of the U.S. National Museum, and for certain nomenclatorial suggestions. My gratitude is also due to Dr. John S. Andrews, Head, Helminthological Investigations, of the same institution and to Dr. George W. Wharton, Head, Department of Zoology, University of Maryland, for providing the necessary facilities. I also have to acknowledge the invaluable help of Mr. Agustin F. Umali, Ichthyologist, Philippine National Museum, for the identification of the fish hosts.

The system of classification by LaRue (1957) is herein adopted for valid reasons.

Order STRIGEATOIDEA LaRue 1926

Suborder BRACHYLAIMATA LaRue 1957

Received for publication May 23, 1958.

Superfamily Bucephaloidea LaRue 1926
Family Bucephalidae Poche 1907

Bucephalus fragilis n. sp.
(Figs. 1, 1a–5)

Host: Megalaspis cordyla (Linnaeus)
Location: intestine
Locality: Navotas, Rizal, Luzon island, Philippines; Malabon, Rizal, Luzon island, Philippines
Prevalence: 12 from 2 of 9 hosts examined.
Type: U.S. Nat. Mus. Helm. Coll. No. 37684
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. Nos. 516 (1)f, 520 (1)f$_2$.
Specific diagnosis: (Based on 10 specimens). Small elongate, delicate, 0.66–0.90 by 0.10–0.23. Cuticle with minute spines. Anterior sucker with 7 tentacles; tentacles rather weak and in most specimens retracted; fairly extended tentacles having broad basal part with slight protuberance and distally small pointed retractile part (Fig. 3). Mouth in midbody, directed anteriorly; pharynx feeble; intestine elongate, saccular, 0.2 by 0.043 in one specimen. Genital organs in posterior third of body in linear arrangement at right side, close to one another. Testes tandem, oblong; cirrus sac elongate, 0.09–0.15 by 0.022–0.04 in 3 specimens; seminal vesicle vesicular; pars prostatica wide and narrowing posteriorly. Genital atrium prominent; genital pore subterminal (Fig. 1a). Ovary small, intestine overlapping it; anterior to anterior testis. Vitellaria in 2 lateral rows of rounded follicles (Fig. 2) about 16 on the right and 15 on the left (one specimen), anterior to uterus between level of pharynx and anterior fourth of body. Uterus ascending to vitellaria almost to anterior end of excretory vesicle and descending beyond genital atrium. Eggs, light yellow, 17–18 microns by 8.6–13.2 microns (Fig. 4). Abnormal eggs showing anopercular protuberance (Fig. 5). Excretory vesicle, saccular, long, extending beyond anteriormost vitellaria.

Discussion: This species resembles *Bucephalus uranoscopi* Yamaguti, 1934 in the position of the mouth, arrangement of vitellaria, relative size and position of the genital organs and extent of excretory vesicle. However, mature specimens of this species are much smaller, the largest worm is less than ¼ the length of *B. uranoscopi*, the rhynchus is less muscular; the intestine shorter and the extent of the uterus and the character of the tentacles are different.

Bucephalus leognathi n. sp.
(Fig. 6)

Host: Leognathus sp.
Location: intestine
Locality: Quiapo market, Manila, Philippines
Prevalence: 4 from 1 of 5 hosts examined.
Holotype: U.S. Nat. Mus. Helm. Coll. No. 37685
Specific diagnosis: (Based on a single specimen; the other 3 were badly mangled). Length 0.68 by 0.30 broad. Anterior sucker 0.09 by 0.07 with tentacles having broad base devoid of protuberances but with retractile pointed tips (Fig. 6). Mouth slightly pre-equatorial, a little anterior to midbody, opposite ovary and just above left testis; pharynx longer than wide; intestine subglobular. Gonads fairly large, in midbody. Testes oval, symmetrical. Cirrus sac 0.23 by 0.068. Genital pore subterminal. Ovary rounded at right side just above the right testis. Uterus extending almost to anterior third of body, not beyond vitellaria. Vitelline follicles rather scattered forming an arch anteriorly. Eggs yellowish, thick-shelled, 17–18 microns by 11.3–13.5 microns. Excretory vesicle not seen.

Discussion: This species closely resembles Nagaty's figures 5 and 9 of *Bucephalus polymorphus* Nagaty (1937) *nec* Baer (1827) in the character of the tentacles and the relative position of the pharynx. However, I agree with Manter (1940) that *B. polymorphus* of Nagaty (1937) is synonymous with *B. varicus* Manter (1940). My species, however, differs from *B. varicus* in the character of the tentacles, position of the testes, extent of uterus, and finer spination. Compared to *B.*

retractilis Yamaguti, 1952, the new species is smaller; the position of its testes and the arrangement of its vitellaria are different and the eggs are larger.

Bucephalus pseudovaricus n. sp.
(Fig. 7)

Host: *Caranx* sp.
Location: intestine and opercular cavity (gills)
Locality: Navotas, Rizal, Luzon island, Philippines
Prevalence: 3 from 2 of 26 hosts examined.
Type: U.S. Nat. Mus. Helm. Coll. No. 37686
Paratype: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 412(2)d_2
Specific diagnosis: (Based on 2 specimens). Body elongate, truncate, 0.98–1.0 by 0.24–0.3. Cuticle spinous. Anterior sucker, 0.077–0.081 by 0.063–0.09 with fairly large, but rather weak tentacles with protuberances (Fig. 7). Mouth at midbody, opposite ovary, just anterior to anterior testis; pharynx usually longer than wide, in one specimen 0.027 by 0.023; intestine globular, sac-like. Gonads fairly large, in midbody. Testes large, globular, oblique; anterior testis overlapping posterior one. Cirrus sac about 1/3 body length, extending slightly beyond anterior testis, enclosing pars prostatica and seminal vesicle; genital pore subterminal. Ovary rounded; pre-testicular, at same level as intestine and partly overlapping proximal end of cirrus sac. Uterus in posterior 2/3rds of body, extending forward slightly anterior to vitellaria. Vitelline follicles in lateral groups, tending to form an arch anteriorly, numbering about 20. Eggs yellowish, thick-shelled, 18–22.5 microns by 12.6–13.5 microns. Excretory vesicle?

Discussion: This species is similar to *Bucephalus varicus* Manter 1940 and *B. priacanthi* Manter, 1940 in general anatomy and the general structure of the tentacles. However, specimens of the above species Nos. 9322 and 36705 respectively, in the U.S. Nat. Mus. Helm. collection were examined for comparison, and were found to differ from the present species in the nature of the sucker, cuticular spines, relative position of the gonads, the relative size of the cirrus sac, extent of the uterus and smaller eggs. *B. retractilis* Yamaguti, 1952 is also similar to the present species which, however, differs in the nature of the tentacles, arrangement of vitellaria, position of testes, extent of cirrus sac and smaller eggs.

Bucephalus paraheterotentaculatus n. sp.
Figs. 8–10

Host: *Seriola nigrofasciata* (Rüppell)
Location: stomach and intestine
Locality: Malabon, Rizai, Luzon island, Philippines
Prevalence: 47 from 3 of 5 hosts examined.
Type: U.S. Nat. Mus. Helm. Coll. 37687
Paratype: University of the Philippines, Dept. of Zoology, Helm. Coll. 554 (1)f
Specific diagnosis: (Based on 10 specimens). Body elongate, subcylindrical, 1.22–4.07 by 0.16–0.5. Cuticle with minute spines. Anterior sucker 0.036–0.072 by 0.072–0.148, with 21 tentacles grouped in multiples of 3 conforming to the basic number of 7 in the genus *Bucephalus* (Fig. 9). Mouth, 0.864–1.728 from anterior end about level of anterior end of cirrus sac, directed downwards. Pharynx 0.026–0.048 by 0.031–0.06. Esophagus rather long, about as long as intestine, but narrow, intestine saccular, 0.15 by 0.085 in type specimen. Seminal vesicle vesicular; prostate cells surrounding pars prostatica and cirrus. Ductus ejaculatorius prominent; genital atrium 0.13–0.24 by 0.08–0.12 (Fig. 10). Testes oval, tandem; anterior testis overlapping posterior testis; latter in contact with anterior end of cirrus sac. Ovary dextral, overlapping anterior testis. Vitellaria consisting of rounded follicles in 2 lateral rows confluent medially in arch fashion, 16 on right and 12 on the left in one specimen. Uterus extending little anterior to vitellaria and not descending beyond genital atrium. Eggs yellowish, 15–26 microns by 11–18 microns. Genital pore subterminal. Excretory vesicle extending to anterior sucker.

Discussion: There are at present 3 named species of *Bucephalus* with more than 7 tentacles, namely, *B. gorgon* (Linton, 1905) Eckmann, 1932 with 24 tentacles, *B. aoria* Verma, 1936 with 14–22 tentacles, and *B. confusus* nov. sp. with 20 tentacles; and in addition, *B. heterotentaculatus* Bravo-Hollis and Sogandares-Bernal, 1954

with the basic number of 7 tentacles but heterogeneous in character, with varied number of basal tentacular projections.

In general anatomy the present species resembles *B. gorgon and B. heterotentaculatus*. However, it differs from *B. gorgon* in the nature and the number of tentacles; posterior and anterior extent of the uterus; number of vitelline follicles and size of eggs. From *B. heterotentaculatus* in the arrangement and broader base of the tentacles and posterior extent of the uterus. *B. aoria* is described as possessing 14–22 tentacles, but the description and illustration do not warrant any certainty for specific comparison, especially of the nature of the tentacles. As far as could be made out, my species differs from *B. aoria* in the position of the gonads; number and arrangement of the vitellaria; anterior extent of the uterus; length of the cirrus sac; and larger eggs.

Kniskern (1952) mentioned the necessity for a restudy of *Bucephalus gorgon* (Linton, 1905) Eckmann 1932. Unfortunately, no specimen of *Gasterostomum gorgon* is available at the U.S. National Museum. However, Linton (1940) figured and redescribed *Gasterostomum gorgon* and named it *Nannoenterum gorgon* (Linton, 1905). Examination of slides of *Nannoenterum gorgon* (Linton, 1940) bearing U.S. Nat. Mus. Helm. Coll. No. 8185 showed the presence of a very definite anterior sucker with tentacles. *Nannoenterum gorgon* (Linton, 1940) therefore should be *Bucephalus gorgon* (Linton, 1940) Eckmann, 1932.

Prosorhynchus crucibulus (Rudolphi, 1819) Odhner, 1905
(Figs. 11–13)
Synonyms: *Monostomum crucibulum* Rudolphi, 1819
Gasterostomum armatum Molin, 1859
(?) *Bucephalus crux* Levinsen, 1881

Host: Sparus berda (Forskäl)
Location: gills and muscles
Locality: (fish ponds), Malabon, Rizal, Luzon island, Philippines
Prevalence: more than 50 from 1 of 2 hosts examined.
Topotype: U.S. Nat. Mus. Helm. Coll. No. 37688; University of the Philippines, Dept. of Zoology, Helm. Coll. No. 539 (2) g.

Specific diagnosis: (Based on 5 immature specimens; measurements from 4). Body elongate, broad, 1.05–1.14 by 0.22–0.3. Cuticle with moderately coarse spines set in quincunx fashion, becoming sparse posteriorly. Rhynchus conical, wedge-shaped, rather large, muscular, measuring 0.24–0.29 by 0.18–0.22. Mouth at end of second third of body; pharynx muscular, 0.043–0.073 by 0.06–0.077 (2 specimens); oesophagus moderately long, 0.043 (1 specimen); intestine saccular, broad and large, 0.27–0.22 (2 specimens). Gonads in posterior ¼th of body. Testes oblique tending to be tandem, globular. Cirrus sac comparatively small, 0.14–0.2 by 0.034–0.05; vesicula seminalis fairly large (Fig. 13); pars prostatica elongate (Fig. 12); genital atrium well developed, with prominent papillae; genital pore subterminal, at end of elongate duct (Fig. 12). Ovary poorly developed, on right, anterior to posterior testis. Uterus without eggs. Vitellaria in 2 lateral rows, in arch formation (one specimen). Vitellaria not well developed (Fig. 11), in 2 lateral rows at level of oesophagus, interrupted opposite gut, and forming single row extending somewhat anterior to intestine. Excretory vesicle? Excretory pore terminal.

Discussion: This species is strikingly similar to *Prosorhynchus crucibulus* (Rudolphi, 1819) Odhner 1905 as described by Caballero, et al (1953) in the shape of the rhynchus, arrangement of vitellaria, nature of pharynx and intestine, position and arrangement of genital organs and cuticular spines. It is about ½ the size of their specimens and the conical rhynchus is more elongate with a more acute inner end. Their measurements of the width of the rhynchus of immature specimens are somewhat greater than those given by Odhner (1905) for this organ in mature worms.

Nicoll (1910) aware of the inadequacy of the description of *P. crucibulus* (Rudolphi, 1819) Odhner, 1905, gave a fairly detailed description of this species based on his materials.

The author agrees with Manter (1953) that *P. squamatus* Odhner 1905 and *P. crucibulus* (Rudolphi, 1819) Odhner, 1905 are different species and that *P. squamatus* should be designated as a valid species and not as a synonym of *P. crucibulus*. Odhner's descriptions and illustrations show that *P. squamatus* has a smaller, oval or lenticular rhynchus while that of *P. crucibulus* is larger and conical. Likewise, Manter (1953) is correct in recognizing as good species *P. costai* Travassos *et al.* (1928) and *P. scalpellus* McFarlane, 1936, contrary to Kniskern's (1952) synonymy. McFarlane's type specimen of *P. scalpellus*, U.S. Nat. Mus. Helm. Coll. No. 9034, has been examined and shows the characters more definitely than do his illustrations.

Prosorhynchus paracrucibulus n. sp.
(Fig. 14)

Host: Ambassis buruensis Bleeker
Location: scales
Locality: Manila Bay, Parañaque, Rizal, Luzon island, Philippines
Prevalence: 4 immature from 1 of 4 hosts examined.
Types and paratypes: U.S. Nat. Mus. Helm. Coll. No. 37689
Specific diagnosis: (Based on 3 specimens). Body broadly elongate, 1.09–1.6 by 0.4–0.48; spinous. Rhynchus cone-shaped with anterior folds, 0.18–0.2 by 0.12–0.2 mm. at greatest breadth, muscular. Mouth in midbody, directed posteriorly; pharynx, 0.1–0.14 by 0.1–0.22; oesophagus well defined, 0.04; gut fairly large, saccular. Gonads in midbody. Testes small, symmetrical in one specimen (Fig. 14), tending to be diagonal in others. Cirrus sac, small, 0.18 by 0.08; vesicula seminalis coiled; pars prostatica narrow. Genital atrium wide; genital pore subterminal. Ovary tiny, dextral, anterior to testes. Vitellaria consisting of about 31 tiny follicles in arch formation, anterior to gut. Uterine coils posterior to right testis and in one specimen between testes; eggs absent. Excretory vesicle? Excretory pore?

Discussion: This species resembles *P. crucibulus* (Rudolphi, 1819) Odhner, 1905 in the relative position of the gut and in the character of the cirrus sac. The rhynchus, however, is very much smaller, more muscular, with anterior folds. Until further details of the life cycle are known, it is tentatively designated as *P. paracrucibulus* nov. sp.

Prosorhynchus luzonicus n. sp.
(Fig. 15)

Host: Lates calcarifer Bloch
Location: intestine
Locality: Malabon, Rizal, Luzon island, Philippines
Prevalence: 3 from 1 of 6 hosts examined
Type: U.S. Nat. Mus. Helm. Coll. No. 376890
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 92g
Specific diagnosis: (Based on 3 specimens; measurements from 2). Body elongate, somewhat pointed at posterior end. Length 1.06–2.02 by 0.27–0.45. Rhynchus conical, muscular, 0.198 by 0.18 (one specimen). Mouth between first and second thirds of body; intestine, saccular; pharynx rather small, diameter 0.043 (1 specimen). Gonads at midbody. Testes globular, tandem; anterior testis smaller than posterior. Posterior testis anterior to cirrus sac, latter large, 0.35 by 0.13 (1 specimen); seminal vesicle partly coiled. Genital pore subterminal. Ovary spherical, small, at pharyngeal level, dextral, in contact with anterior testis but not overlapping it. Vitellaria in 2 widely separated lateral groups about 13 on the right and 15 on the left; follicles rounded, well defined, extending from rhynchus almost to anterior testis. Uterine coils lying between ovary and genital pore. Egg yellowish, 30–39 microns by 17–24 microns. Excretory pore terminal. Extent of excretory vesicle unknown.

Discussion: This species very closely resembles *P. atlanticus* (Syn. *P. pacificus* Manter, 1940) Manter, 1940 and was collected from related hosts belonging to the family Serranidae.

Comparative measurements in microns of eggs of the 3 species are as follows: *P. pacificus*, 24–27 by 12–14 (type specimen, Manter); *P. atlanticus* 27–34 by 14–22 (Manter); *P. luzonicus* n. sp. 30–39 by 17–24 Velasquez. The egg of the present species is very much larger than that of *P. pacificus* and *P. atlanticus* and I therefore propose to designate it as a new species, *P. luzonicus*.

Prosorhynchus longus nov. sp.
(Fig. 16)

Host: Psettodes erumei (Bloch and Schneider)
Location: stomach
Locality: Divisoria market, Manila, Philippines
Prevalence: 7 immature from 1 of 9 hosts examined
Type: U.S. Nat. Mus. Helm. Coll. No. 37691
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 75d
Specific diagnosis: (Based on 5 immature specimens). Body relatively long, cylindrical, 0.58–1.64 by 0.196–0.24 with long neck; cuticular spines minute. Rhnchus weakly muscular, lenticular, plug-shaped, 0.1–0.12 by 0.12–0.14. Mouth at distal third of body, usually directed posteriorly, but anteriorly in some. Pharynx muscular, 0.043–0.06 by 0.044–0.047; oesophagus, 0.043 long; intestine, elongate, saccular, 0.47 long in one specimen. Gonads in posterior third of body. Testes tandem, globular; cirrus sac short, small about 1/10 body length, 0.24–0.34 by 0.04–0.09. Seminal vesicle subglobular; pars prostatica narrow; genital atrium widened; genital pore subterminal. Ovary approximately 1/3 diameter of testis. Vitellaria 26, arranged in 2 lateral rows, posterior to ovary; in 1 specimen grouped in midbody partly overlapping anterior and posterior testis (Fig. 16); uterus not well developed. Excretory pore? Excretory vesicle?

Discussion: This species resembles *Prosorhynchus freitasi* Nagaty, 1937 and *P. longicollis* Yamaguti, 1953 in general anatomy and relative body size. It differs from *P. longicollis* in position of vitellaria; shape and length of intestine; position of gonads and direction of the mouth; from *P. freitasi* in the arrangement of vitellaria; length of intestine; and position of the gonads.

Rhipidocotyle eggletoni n. sp.
(Figs. 17–18)

Hosts: Sillago sihama (Forskäl); *Gazza minuta* (Bloch)
Location: muscles
Locality: Manila Bay, Luzon island, Philippines
Prevalence: 6 immature from 3 of 5 hosts examined.
Type: U.S. Nat. Mus. Helm. Coll. No. 37692
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 494(2)g.
Specific diagnosis: (Based on 4 immature specimens). Body elongate, truncate, 0.98–1.98 by 0.14–0.2. Cuticle with fine spines. Rhynchus 0.077–0.09 by 0.072–0.091 with hood, dome-shaped in 1 specimen, notched medially at more anterior convex side; front view, more posterior border with short, conical, rather small papillae, 1 short median and 2 lateral. Hood when wrinkled not showing papillae (Fig. 18). Mouth in midbody; pharynx weak, 0.032–0.041 by 0.023–0.045 (2 specimens); esophagus relatively long, almost as long as intestine. Gonads at junction of median and last thirds of body, dextral in 1 specimen, median in type. Testes tandem; cirrus sac, 0.16–0.25 by 0.028–0.045 at posterior ¼th of body; seminal vesicle ovoid; pars prostatica narrow; genital pore subterminal. Ovary overlapping intestine (1 specimen), anterior to anterior testis; uterus without mature eggs, limb ascending level of pharynx and then descending to genital atrium. Vitellaria (1 specimen) in lateral fields at level of testes. Excretory pore terminal. Excretory vesicle in posterior 4/5th of body.

This species is named after my former Professor, Dr. Frank E. Eggleton, Dept. of Zoology, University of Michigan, Ann Arbor, Michigan.

Discussion: This species resembles *Rhipidocotyle kahlili* Nagaty, 1937 and *R. adbaculum* Manter, 1940 in general anatomy but differs from *R. kahlili* in the character of the hood; extent of the excretory vesicle and relative size and extent of uterus. It differs from *R. adbaculum* in the nature of the hood; more elongate body; and more posterior position of the genital organs. Because of immaturity,

the extent of the uterus and the size of the eggs cannot be used for specific determination. *Chanos chanos*, the host of *R. kahlili*, abounds in Philippine waters. My specimens though immature present certain useful characters of specific significance. Until the life cycle is known, I am considering the present species as new.

Rhipidocotyle laruei n. sp.
(Figs. 19–20)

Host: Psettodes erumei (Bloch and Schneider)
Location: stomach and intestine
Locality: Malabon, Rizal, Luzon island, Philippines
Prevalence: 5 mature and 1 immature from 1 host of 9 examined
Type: U.S. Nat. Mus. Helm. Coll. No. 37693; paratype No. 37694
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 540 (1) f.
Specific diagnosis: (Based on 2 specimens). Body truncate, elongate, 1.0–1.15 by 0.25–0.28. Neck-like region well formed (one contracted specimen). Body with rather coarse, spines scale-like, arranged in transverse rows, more closely set anteriorly and more sparse posteriorly. Anterior sucker subterminal, with hood hardly discernible in side view; but seen in front view, hood provided with median and 2 lateral papillae (probably a total of 7) making a sort of polygonal cap (Fig. 20). Mouth at midbody; pharynx subglobular; intestine saccular, directed anteriorly. Gonads in midbody. Testes tandem; anterior testis smaller than posterior and overlapping. Cirrus sac (type) 0.4 by 0.08, almost ½ body length, extending to anterior border of posterior testis. Seminal vesicle coiled; pars prostatica elongate, wider anteriorly. Genital atrium well developed; genital pore subterminal. Ovary rounded, slightly anterior to anterior testis. Uterus extending to anterior groups of vitellaria. Vitelline follicles in 2 lateral rows, 9–10 on the left side and 13–15 on the right, in type specimen compressed, tending to be confined at median field. Excretory vesicle elongate, extending to level of gut; excretory pore terminal.

Discussion: I agree with Hopkins (1950) (mimeographed) that under certain circumstances, diagnosis is difficult. The specimens must be properly fixed and oriented in order to see the hood. However, fortunately, 1 specimen of this species was properly oriented with the cap rather well spread (Fig. 19). This species resembles *Rhipidocotyle longleyi* Manter, 1934 in general anatomy but differs from it in size of body, length of cirrus sac, position of genital atrium, extent of vitellaria, character of the papillae of the hood and cuticular spines.

Manter (1934) noted the resemblance of *R. longleyi* with *R. baculum* (Linton, 1905) Eckmann, 1932. I have noted, however, that the latter species presents much confusion. It was first described by Linton (1901) as *Gasterostomum* sp. from *Scomberomorus maculatus* (U.S. Nat. Mus. Helm. Coll. 6672). The description was based on poorly preserved specimens but he later (Linton, 1905) named the species *Gasterostomum baculum*, and presented new figures. Examination of Linton's unstained specimens (U.S. Nat. Mus. Helm. Coll. 6672) showed an anterior sucker with a cap. Nicoll (1915) wrongly placed this species in the genus *Bucephalus*. Manter (1931) made a new combination, *Nannoenterum baculum* (Linton, 1905) Manter, 1931. Subsequently, Eckmann (1932) on the basis of Manter's specimens taken from the same host, transferred the species from *Nannoenterum* Ozaki, 1924 to *Rhipidocotyle* Diesing, 1858, the species then becoming *Rhipidocotyle baculum* (Linton, 1905) Eckmann, 1932. Without noting Eckmann's synonymy Linton (1940), using new material collected from several hosts, described and figured a species which he erroneously identified as that which he had described in 1901 and 1905 and named the new material *Nannoenterum baculum*. Manter (1947) referred Linton's material described in 1940 to *Rhipidocotyle baculum* (Linton, 1905) Eckmann, 1932, a name which was correct for the material reported in 1901, 1905 by Linton. Examination of Linton's specimens reported in 1940 and

deposited in the U.S. Nat. Mus. Helm. Coll. Nos. 8180–8185 from *Sphyraena borealis, Decapterus macarellus, Menticirrhus saxatilis, Raja diaphanes* and *Raja laevis* showed that these worms possessed tentacles; his descriptions indicate 20 tentacles, his illustrations fewer. But, unluckily, he said that the one from *Scomberomorus maculatus* (type host) was imperfect; yet he indicated that he saw tentacles in it. It is apparent that Linton (1940) was not unaware of the fact that he was not dealing with *baculum*. Likewise, Manter (1947) may have presumed that Linton's material described in 1940 was that reported in 1901, 1905. I therefore propose that one of the lectotypes of Linton's *G. baculum* (unmounted specimens) described in his paper (1901, 1905) be designated as syntype for *Rhipidocotyle baculum* (Linton, 1905) Eckmann, 1932.

No additional species of gasterostomes has been reported from the above hosts for Linton's misidentified specimens (1940) with tentacles, described and figured by him as *Nannoenterum baculum* and which Manter (1947) referred to as *Rhipidocotyle baculum* (Linton, 1905) Eckmann 1932. I propose a new name *Bucephalus confusus* for the misdetermined specimens with tentacles described and figured in 1940 as *Nannoenterum baculum* Linton (See Linton, 1940: pp. 33–36; Figs. 252–253.

Separated out from the U.S. Nat. Mus. Helm. Coll. No. 6672 (unstained specimens) from *Scomberomorus maculatus* is 1 specimen (38337) which is without a hood but having a sucker lacking tentacles and resembling that figured by Linton (1905) as *Gasterostomum arcuatum* (Linton, 1900). This species was renamed *Bucephalopsis arcuata* (Linton, 1900) Eckmann ,1932. Hopkins (1954) proposed that *Bucephalopsis* be restricted only to a cercaria, *B. haimeanus* Lacaze-Duthiers, 1854 whose life history is not known, and further that all other species formerly included in the genus *Bucephalopsis* be designated as *Bucephaloides*. Therefore, *Bucephalopsis arcuata* (Linton, 1900) Eckmann, 1932 becomes *Bucephaloides arcuatus* (Linton, 1900) new comb. The specimen (38337, U.S. Nat. Mus.) is now entered as *Bucephaloides arcuatus* (?) (Linton). Since the only specimen is unstained, the specific determination is not certain.

Bucephaloides philippinorum n. sp.
(Figs. 21–22)

Host: Sphyraena langsar Bleeker
Location: Stomach and gills?
Locality: Quiapo market, Manila, Philippines
Prevalence: 3 from 1 host of 7 examined.
Type: U.S. Nat. Mus. Helm. Coll. No. 37695
Paratype: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 66a.
Specific diagnosis: (Based on 3 specimens). Body elongate, narrow, anteriorly, posterior end rounded, 2.44–2.68 by 0.28–0.44. Cuticle with minute spines. Anterior sucker, 0.08–0.11 by 0.09–0.10; hood rhynchus and tentacles absent. Mouth in midbody in 2 specimens, in anterior third of body in type. Pharynx moderately large in 2 specimens, 0.06–0.11 by 0.06. Esophagus short, intestine elongate, saccular, reaching to midbody (type), 0.76 long. Gonads in posterior third of body. Testes tandem, flask-shaped; cirrus sac elongate, narrow, 0.54–0.76 by 0.06-0.08; seminal vesicle small, vesicular; pars prostatica narrow, long; genital atrium wide; genital pore subterminal. Ovary small, anterior to anterior testis; uterus extending into anterior third of body, farther than anterior extent of vitellaria. Vitelline follicles large, ovoid, flattened, close together, arranged into 2 lateral rows numbering 19 on right side and 12 on left. Eggs yellowish, 15–17 micrins by 11–13 microns. Excretory pore terminal; excretory vesicle?

Discussion: This species resembles *Bucephalopsis tenuis* Yamaguti, 1952 and *B. exilis* Nicoll, 1915. It differs from the former in the more anterior position of the

pharynx; more elongate, saccular intestine; arrangement and number of vitelline follicles; relative position of gonads; and anterior and posterior extent of uterus; and smaller eggs. From *B. exilis*, it differs in the more elongate body; relative position of pharynx; less anterior extent of uterus; number of vitelline follicles and smaller eggs.

As stated in the preceding discussion I agree with Hopkins (1954) that all species of adults belonging to *Bucephalopsis* Lacaze-Duthiers, 1854 should be assigned to *Bucephaloides* Hopkins, 1954. Hence *Bucephaloides tenuis* Yamaguti is designated as *Bucephaloides tenuis* (Yamaguti, 1952) new comb., likewise, *Bucephalopsis exilis* Nicoll, 1915 should be *Bucephaloides exilis* (Nicoll, 1915) new comb.

Alcicornis cirrudiscoides n. sp.
(Figs. 23–25)

Host: Caranx sp.
Location: intestine
Locality: Navotas, Rizal, Luzon island Philippines.
Prevalence: 5 from 4 of 26 hosts examined.
Type: U.S. Nat. Mus. Helm. Coll. No. 37696
Paratypes: University of the Philippines, Dept. of Zoology, Helm. Coll. Nos. 409(1)f, 412-(2)d_2

Specific diagnosis: (Based on 2 specimens). Body elongate, truncate, 0.54–0.67 by 0.12–0.13. Cuticular spines minute. Rhynchus wedge-shaped with 7 tentacles, tentacular prongs single, prominent (Fig. 25). Mouth at midbody, directed posteriorly; pharynx small, weakly muscular; intestine small, saccular. Gonads at midbody. Testes posterior to pharynx; anterior testis oblong, posterior one elongate, sausage-shaped. Cirrus sac elongate, 0.13–0.2 by 0.022, anterior end peculiarly shaped, concave, disc-like into which vas deferens enters to join the vesicula seminalis. Genital atrium elongate, provided with prominent papillae; muscular duct leading to genital pore (pushed out by pressure); pars prostatica long; seminal vesicle sub-globular (Fig. 24). Ovary small, anterior to and overlapping anterior testis, overlapped by intestine. Uterus occupying much of body, extending anteriorly to extreme limits of vitellaria, posteriorly not beyond genital atrium. Vitelline glands about 11 on the right and 15 on the left. Eggs thick-shelled, 34–39 microns by 21–26 microns.

Discussion: Only 3 other species of *Alcicornis* have been described. *A. carangis* MacCallum, 1917 from *Caranx ruber*, Florida; *A. baylisi* Nagaty 1937 from *Caranx* sp., Red Sea and *Caranx equula*, Okinawa; and *A. longicornutus* Manter 1954 from *Kathetostoma giganteum*, New Zealand.

All species except the last show acute conical rhynchus with well defined tentacles provided with small basal prongs. My species differs from all other described species in the size of the rhynchus, nature of tentacular prongs (Fig. 25), the relative position of the cirrus sac with its peculiar discoidal anterior end; position of the gonads; shape of posterior testis; extent of vitellaria and uterus.

Neidhartia mcintoshi n. sp.
(Fig. 26)

Host: Epinephelus bleekeri (Vaillant and Bocour)
Location: muscle, stomach and intestine
Locality: Malabon, Rizal, Luzon island, Philippines
Prevalence: 2 mature out of 18 from 4 of 14 hosts examined.
Type and paratype: U.S. Nat. Mus. Helm. Coll. No. 37697
Paratype: University of the Philippines, Dept. of Zoology, Helm. Coll. No. 416(1)f_2.

Specific diagnosis: (Based on 2 mature and 4 immature worms). Measurements were made from 1 fairly extended specimen and 1 partly contracted mature worm. Length 0.82–1.0 by 0.25–0.26 wide. Immature worms 0.49–0.61 by 0.11–0.2. Body elongate, more or less truncate. Cuticle with minute spines. Rhynchus cone-shaped with expanded convex anterior surface; in one specimen measuring 0.17 by 0.14. Mouth at midbody; pharynx prominent but feebly muscular; intestine saccular. Testes tandem, dextral, separated by uterus and ovary. Anterior testis

at level of ovary just posterior to intestine. Cirrus sac rather muscular, 0.3 by 0.09 (one specimen), about 1/3 body length; pars prostatica connected anteriorly to prominent seminal vesicle. Genital atrium fairly large; genital pore subterminal. Ovary globular, sinistral, opposite testes, but much larger. Uterus with few eggs extending anterior to posterior testis and posteriorly not beyond genital atrium. Vitelline follicles in 2 lateral rows, 12 on right and 13 on left, meeting medially in arch formation. Eggs yellowish, 26–34 microns by 17–26 microns. Excretory pore terminal. Excretory vesicle extending to rhynchus?

Discussion: There are at present 5 known species of *Neidhartia*. *N. neidharti* Nagaty, 1937, *N. ghardagae* Nagaty, 1937, *N. synodi* (Yamaguti, 1938) Manter, 1940, *N. microrhyncha* Chauhan, 1943 and *N. polydactyli* Manter, 1953. The present species occurs as metacercaria and adult in the same host, showing evidence that infection of one fish is brought about possibly through the eating of the smaller fish by the larger. The present species differs from all of the above named species in the position of the mouth and pharynx; relative size of smooth ovary and testis; the shape and less muscular nature of rhynchus; and extent of uterus. *N. synodi* (Yamaguti, 1938) Manter, 1940 does not seem to fit in the genus *Neidhartia*. Although the position of the ovary may place it in this genus, yet the rhynchus is lenticular rather than conical, as in *Neidhartia*. Manter (1953) has recognized 2 types of *Prosorhynchus*: one with an oval or lenticular rhynchus and the other conical. *Neidhartia* Nagaty, 1937 has a conical rhynchus with ovary at left. *N. synodi* (Yamaguti, 1938) Manter, 1940 has the oval or lenticular rhynchus and the ovary is dextral and in between the 2 testes. There is reason to believe that this species should belong to the genus *Prosorhynchus* on the basis of the position of the testes and the nature of the rhynchus, except that the ovary is in between the two testes and is dextral. Hence, for the present *Pseudoprosorhynchus synodi* Yamaguti, 1938 should stand as a valid species until more material is available.

SUMMARY

1. Thirteen species in 6 genera of the family Bucephalidae from Philippine food fishes are described, 12 of which are considered new to science.

2. *Prosorhynchus crucibulus* (Rudolphi, 1819) Odhner, 1905 is herein reported from a new host and locality.

3. One of the lectotypes of Linton's *Gasterostomum baculum* (unmounted specimens) described in his paper (1901, 1905) has been designated as syntype for *Rhipidocotyle baculum* (Linton, 1905) Eckmann, 1932.

4. The misidentified specimens with tentacles described and figured by Linton (1940) as *Nannoenterum baculum* are here named *Bucephalus confusus* nom. nov.

5. The single specimen separated out from the U.S. Nat. Mus. Helm. Coll. 6672 is now entered as *Bucephaloides arcuatus* (?) (Linton, 1900) n. comb.

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EXPLANATION OF PLATE

(All figures drawn with the aid of a camera lucida)

FIGURE 1. Ventral view of *Bucephalus fragilis* n. sp.

FIGURE 1a. Posterior view of *Bucephalus fragilis* showing the genital atrium and a portion of the cirrus sac.

FIGURE 2. Anterior view of another specimen of *Bucephalus fragilis* showing the arrangement of the vitellaria and extent of the saccular excretory vesicle.

FIGURE 3. Tentacles of *Bucephalus fragilis* with small basal protuberances.

FIGURE 4. Normal eggs of *Bucephalus fragilis*.

FIGURE 5. Abnormal eggs of *Bucephalus fragilis* with anopercular protuberances.

FIGURE 6. Ventral view of *Bucephalus leognathi* n. sp. with the tentacles devoid of basal protuberances but with retractile pointed tips.

FIGURE 7. Ventral view of *Bucephalus pseudovaricus* n. sp.

FIGURE 8. Ventral view of *Bucephalus paraheterotentaculatus* n. sp.

FIGURE 9. Anterior ventral view of *Bucephalus paraheterotentaculatus* showing the nature and arrangement of the tentacles.

FIGURE 10. Posterior region of *Bucephalus paraheterotentaculatus* showing the genital atrium and a portion of the cirrus sac.

FIGURE 11. Ventral view of an immature *Prosorhynchus crucibulus* (Rudolphi, 1819) Odhner 1905.

FIGURE 12. Posterior region of *Prosorhynchus crucibulus* showing the genital atrium and a part of the cirrus sac.

FIGURE 13. Anterior portion of the cirrus sac of *Prosorhynchus crucibulus* showing the vas deferens, vesicula seminalis and a portion of the pars prostatica.

FIGURE 14. Ventral view of *Prosorhynchus paracrucibulus* n. sp.

FIGURE 15. Ventral view of *Prosorhynchus luzonicus* n. sp.

FIGURE 16. Ventral view of *Prosorhynchus longus* n. sp. Note vitellaria grouped in mid-body partly overlapping anterior and posterior testes.

FIGURE 17. Ventral view of *Rhipidocotyle eggletoni* n. sp. showing the nature of the hood.

FIGURE 18. Another specimen of *Rhipidocotyle eggletoni* showing the wrinkled hood with the papillae not discernible.

FIGURE 19. Ventro-lateral view of *Rhipidocotyle laruei* n. sp. showing general anatomy and slightly discernible hood.

FIGURE 20. Anterior frontal view of a contracted specimen of *R. laruei* showing the nature of the hood and the papillae.

FIGURE 21. Ventro-lateral view of *Bucephaloides philippinorum* n. sp. with long distended gut and anterior sucker without tentacles.

FIGURE 22. Another specimen of *B. philippinorum* showing the very much contracted gut.

FIGURE 23. Ventral view of *Alcicornis cirrudiscoides* n. sp.

FIGURE 24. *Alcicornis cirrudiscoides*, ventro-lateral view of the cirrus sac under oil immersion showing the disc-like anterior end, vas deferens vesicula seminalis, pars prostatica and genital atrium. Note the muscular duct leading to genital pore pushed out by pressure.

FIGURE 25. Tentacles of *Alcicornis cirrudiscoides* showing the character of the basal prongs.

FIGURE 26. Ventral view of *Neidhartia mcintoshi* n. sp. Note the relative position of the gonads and the sinistral ovary.

RESEARCH NOTE

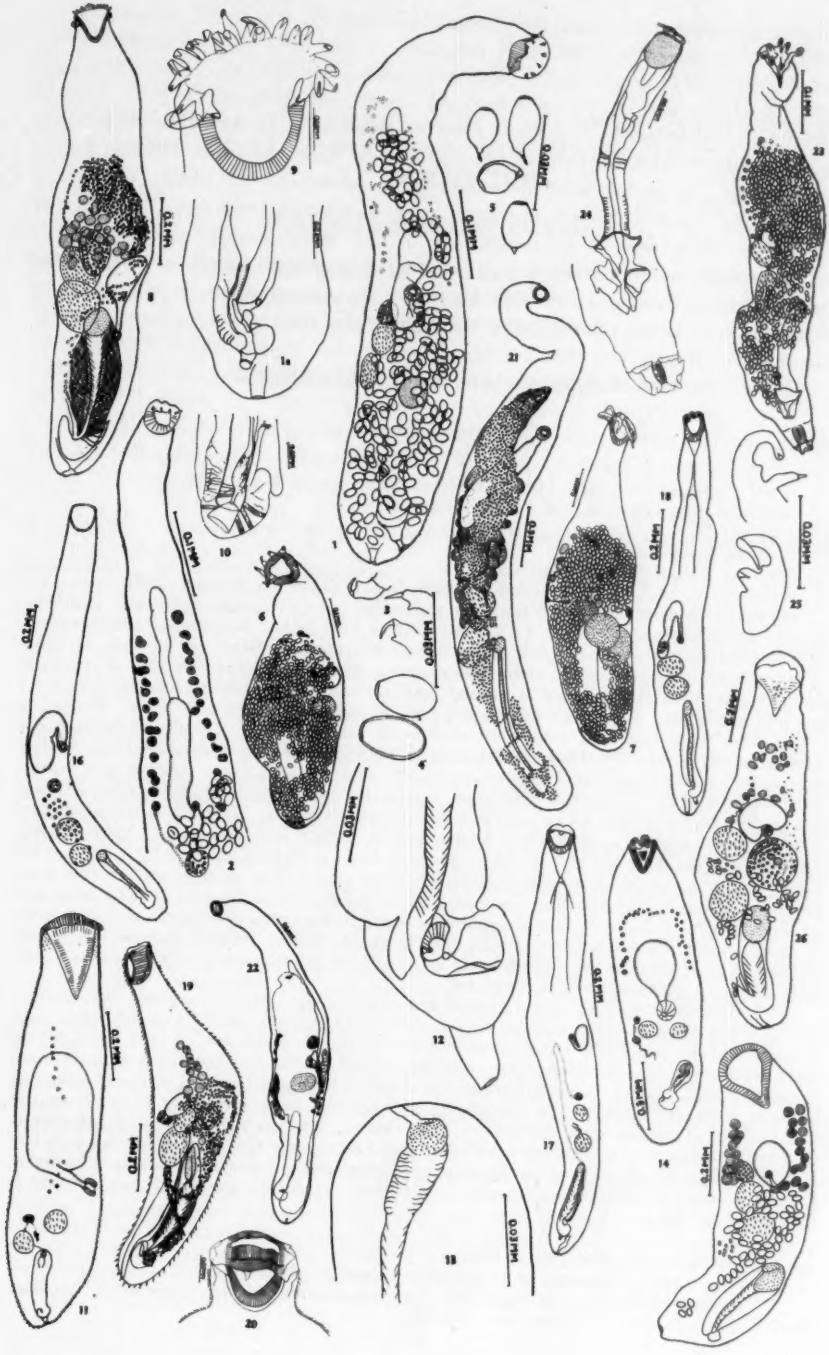
STAGNICOLA (HINCKLEYIA) CAPERATA (SAY), A NATURAL INTERMEDIATE HOST FOR *FASCIOLOIDES MAGNA* (BASSI, 1875), IN MINNESOTA

In a previous note, (1955, J. Parasitol. 41: 115) *Stagnicola palustris* (Müller), was reported to serve experimentally as an intermediate host for *Fascioloides magna* in Minnesota.

During the summer of 1955, while examining molluscs as potential intermediate hosts for this trematode, one naturally infected snail from a pasture in Pine County, Minnesota, yielded fasciolinid cercaria. The snail shed cercariae on June 15. After 12 days of encystment on the side of a glass sputum vial, 20 metacercariae were removed with a fine camel hair brush, mixed with ground feed in a small gelatin capsule and fed to a guinea pig. The guinea pig died 5 weeks after the experimental feeding. Upon necropsy, one immature fluke, 4.00 × 3.00 mm, was recovered from the liver parenchyma.

The snail was identified as *Stagnicola (Hinckleyia) caperata* (Say). This is the first time this species has been involved as an intermediate host for *F. magna* in North America. From its size and "weather-beaten" appearance, this snail had certainly "wintered over" at least one winter. Since the metacercariae were shed on June 15, it seems improbable in this geographical area, that this individual had been infected the same spring that the metacercariae were shed. It seems more likely it was infected the previous autumn and that the larval flukes were present in the intermediate host throughout its period of winter inactivity. If such does occur, it is of economic importance in that *F. magna* infections may be picked up early in the grazing season in Minnesota.

The identification of this mollusc, as well as others by Dr. J. P. E. Morrison, Division of Molluscs, U. S. National Museum, is gratefully acknowledged. His interest and kind help is much appreciated.—H. J. GRIFFITHS, *Division of Pathology and Parasitology, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota.* This note has been approved as Scientific Journal Series Paper No. 4036, Minnesota Agricultural Experiment Station.



CLEPTODISCUS KYPHOSI, A NEW TREMATODE (PARAMPHISTOMATIDAE) IN *KYPHOSUS SECTATRIX* (LINN.) FROM BIMINI, B. W. I.*

FRANKLIN SOGANDARES-BERNAL**

The species described below was collected during the summer of 1957 at the Lerner Marine Laboratory of The American Museum of Natural History. The study was done at the University of Nebraska under the supervision of Dr. H. W. Manter.

Unless otherwise cited, all measurements are in millimeters.

Cleptodiscus kyphosi n. sp.
(Figs. 1 to 3)

Host: Kyphosus sectatrix (Linn.); bermuda chub; family Kyphosidae.
Incidence of infection: In 1 of 4 hosts.
Number: 14.
Location: Mid-intestine.
Locality: Bimini, British West Indies.
Holotype: U. S. National Museum Helminthological Collection Number 38571.

Diagnosis (measurements on 6 favorable specimens): Body elongate, cylindrical, with conspicuous cuticular rugations, 3.2 to 5.2 long by 0.817 to 0.912 wide. Acetabulum ventroterminal, 0.494 to 0.551 long by 0.513 to 0.665 wide. Buccal aperture at anterior end of body, terminal or subterminal. Prepharyngeal atrium present or not, depending upon contraction of specimen. Pharynx without an anterior band of circular muscles; with 2 lateral diverticula, from ¼ to ½ length of esophagus. Gland cells surrounding base of pharynx between diverticula. Esophagus thick-walled, connecting with a spherical esophageal bulb consisting of both longitudinal and circular muscle fibers; bulb 0.304 to 0.361 long by 0.475 to 0.513 wide. Gland cells surrounding esophagus and esophageal bulb. Ceca ending blindly at posterior ¾ body. Genital pore ventral and sinistral to mid-line at cecal bifurcation, surrounded by muscles and gland cells; followed by a shallow genital atrium with a central, muscular, genital papilla. Testes 2, spherical, a short distance posterior to cecal bifurcation, tandem and in contact with each other; anterior testis 0.361 to 0.475 long by 0.475 to 0.513 wide; posterior testis 0.380 to 0.532 long by 0.456 to 0.513 wide. Cirrus sac spherical, extending diagonally from genital pore to very near or in contact with anterior border of foretestis. External seminal vesicle located between anterior testis and cecal bifurcation, coiling 3 or 4 times, connecting with coiled internal seminal vesicle in posterior ¼ of cirrus sac. Small prostatic vesicle present at junction of internal seminal vesicle and swelling of an ejaculatory duct which opens externally on genital papilla. Prostate gland cells surrounding all structures within cirrus sac.

Ovary spherical, on inner aspect of blind end of left cecum. Mehlis' complex slightly overlapping ovary dorsally on its posterior border. Uterus extending posterior to ovary for a short distance, between ovary and testes, ascending intercecally on right side of both testes, filling pretesticular area to cecal bifurcation; entering genital papilla and opening separately from ejaculatory duct. Large vitellaria irregular in shape, extending from level of ovary almost to posterior testis, extracecal and/or overlapping each cecum. Eggs near ovary 56 to 72 microns long by 32 to 35 microns wide, operculated, thick-shelled. Excretory pore dorsal to acetabulum. Excretory bladder median between acetabulum and ovary; collecting tubules one on each side of body, ending blindly at anterior level of esophageal bulb. Lymphatic vessels voluminous, forming a branched lymph sinus dorsal to acetabulum; extending one on each side of body to join dorsal to pharynx.

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* Studies from the Department of Zoology, University of Nebraska, No. 305.
** Present address: Florida State Board of Conservation Marine Laboratory, Maritime Base, Bayboro Harbor, St. Petersburg, Florida.

Discussion: The genus *Cleptodiscus* Linton, 1910 contains the following species: *C. reticulatus* Linton, 1910 and *C. bulbosus* Hanson, 1955. Yamaguti (1953) placed the genus *Neocladorchis* Bhalerao, 1937, in synonymy with *Cleptodiscus.* Hanson (1955) accepted this view. *Neocladorchis poonaensis* Bhalerao, 1937 (type species), possesses 3 lymphatic vessels on each side of the body, as compared with 1 lymphatic vessel on each side of the body in *C. reticulatus, C. bulbosus,* and *C. kyphosi.* Until the significance of the number of branches of the lymphatic system is better understood, I prefer to retain the genus *Neocladorchis.*

Cleptodiscus kyphosi differs from *C. reticulatus* and *C. bulbosus* by possessing tandem anterior testes instead of testes oblique and more posterior, uterus dextral to testes instead of intertesticular, and sinistral rather than central genital pore. *Cleptodiscus kyphosi* further differs from *C. reticulatus* by possessing a spherical esophageal bulb as compared with esophageal bulb absent (replaced by a tube), and from *C. bulbosus* by lacking a strong band of sphincter muscles surrounding anterior border of pharynx.

Sagittal sections of *C. kyphosi* show that the ceca possess either cilia or microvilli, probably the latter. These structures are at present being studied further.

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RESEARCH NOTE

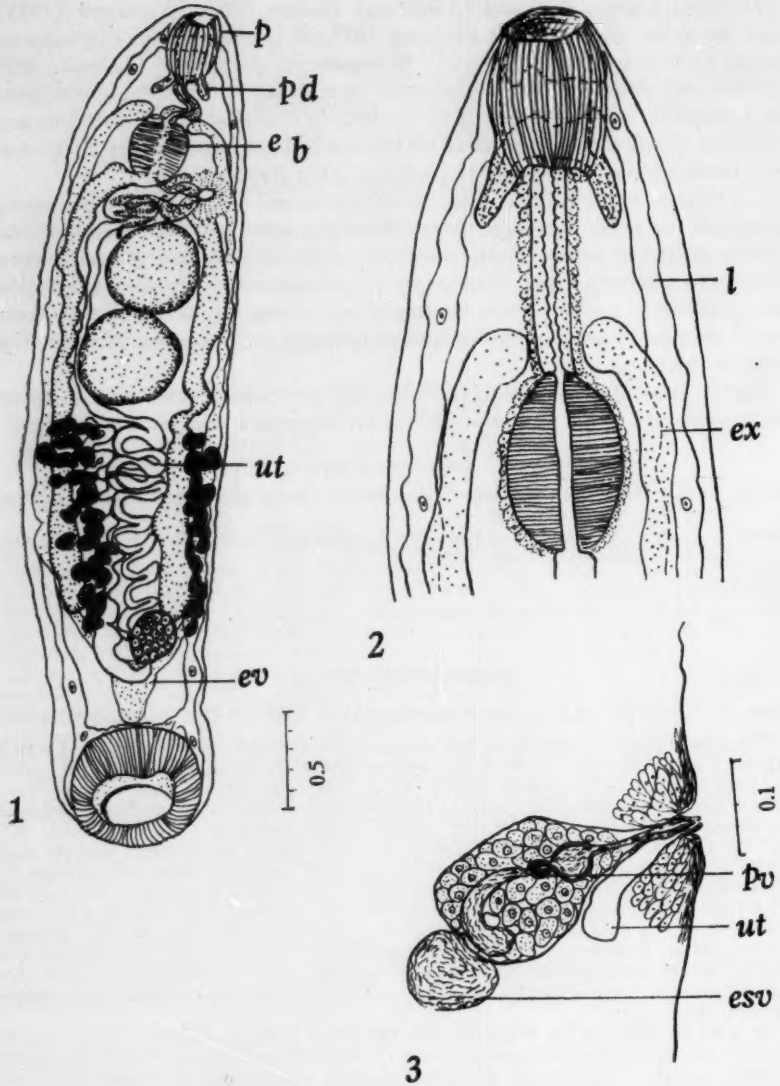
THE STATUS OF THE ACANTHOCEPHALAN GENUS *CENTRORHYNCHUS*

The generic name *Centrorhynchus* was proposed by Luehe (1911, Süsswasserfauna Deutschlands **16:** 1–116). Recently this name was declared invalid by Dollfus and Golvan (1957, Bull. Inst. Franc. Afrique Noire, serie A. **19:** 412–416). They noted that the name *Centrorhynchus* had been assigned to a coleopteran insect in 1829, according to Neave's *Nomenclator Zoologicus.* The quotation from Neave is as follows: "*Centrorhynchus* Steven or Fischer Waldheim 1829, Mus. nat. Univ. Caesareae Mosquensis, **99** [*n. n.*]—Col." After the discovery that the name *Centrorhynchus* was preoccupied, Dollfus and Golvan (1957) proposed the name *Gordiorhynchus* Meyer, 1931, to replace *Centrorhynchus* Luehe, 1911. Meyer (1931, Zool. Jahrb, Abt. Syst. **62:** 53–108) had proposed the genus *Gordiorhynchus* for some Acanthocephala from an owl. These parasites showed a pronounced pseudosegmentation, but in all other respects they resembled *Centrorhynchus.* Witenberg (1932, Boll. Zool. **3:** 253–266) recognized the close relationship of these two genera and placed *Gordiorhynchus* in synonymy with *Centrorhynchus.* This seems reasonable since pesudosegmentation occurs in a number of genera and can hardly be considered of generic significance.

In a review paper on the Acanthocephala the writer (1951, J. Tennessee Acad. Sci. **26:** 282–311) listed *Gordiorhynchus* as a synonym of *Centrorhynchus,* following Witenberg's opinion. I failed to state that Witenberg was the first to recognize this synonymy and should like to take this opportunity to give him credit for it.

Later in 1957 the genus *Centrorhynchus* Luehe, 1911, was restored as a valid name by Dollfus. It was found that the homonymy had been caused by a typographical error (Dollfus, 1957, Arch. Inst. Pasteur Maroc., Misc. Helm. Maroc. **5:** 403–407). In the original publication, Museum Historiae Universitatis Caesareae Mosquensis, 1829, the generic name for the coleopteran was given as *Ceutorynchus* Germar, 1824. The spelling of this name was later changed to *Ceuthorhynchus* by Agassiz in 1846. Therefore, *Centrorhynchus* Luehe, 1911, is a valid acanthocephalan genus, and *Gordiorhynchus* Meyer, 1931, should be regarded as a synonym.—HELEN L. WARD, *Department of Zoology and Entomology, University of Tennessee, Knoxville.*

PLATE I



EXPLANATION OF FIGURES

Figures 1 and 3 were drawn with the aid of a camera lucida. The projected scale has value in millimeters. Abbreviations used: EB, esophageal bulb; ESV, external seminal vesicle; EV, excretory vesicle; EX, excretory canal; L, lymphatic vessel; P, pharynx; PD, pharyngeal diverticulum; PV, prostatic vesicle; UT, uterus.

FIGURE 1. *C. kyphosi,* ventral view.

FIGURE 2. Schematic diagram of anterior end of *C. kyphosi,* ventral view.

FIGURE 3. Sagittal section of *C. kyphosi,* showing terminal genitalia.

STUDIES ON THE HELMINTHS OF NORTH CAROLINA VERTEBRATES. I. *PROCYOTREMA MARSUPIFORMIS* N. G., N. SP. (STRIGEIDA: DIPLOSTOMATIDAE) FROM THE PANCREATIC DUCT OF THE RACCOON*

REINARD HARKEMA AND GROVER C. MILLER

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The examination of a raccoon, *Procyon lotor lotor,* collected in Wake County, N. C. on December 15, 1957, revealed an enlarged pancreas due to markedly hypertrophied pancreatic ducts, which contained 86 strigeid trematodes. Several of the specimens were attached by the holdfast. Hemorrhagic host tissue was present in the cavity between the ventral body wall and holdfast.

Several years earlier a limited amount of material of this trematode had been obtained from formalin-preserved viscera of raccoons from Pasquotank County, N. C. The specimens constitute a new genus and species of the Strigeida and are assigned to the Family Diplostomatidae.

Procyotrema n. g.

Generic Diagnosis: Family Diplostomatidae: sub-family Alariinae. Body large, elongated, subcylindrical, forebody longer and slightly narrower than hindbody. Holdfast organ unlobed, large, extending posteriorly from the region of the ventral sucker to the posterior limit of forebody; contained in an elongated pouch formed by the ventral wall of the forebody. Vitellaria follicular, restricted to forebody. Ovary at anterior end of hindbody. Mehlis' gland and seminal reservoir just posterior to ovary, approximately median, anterior to or between anterior ends of testes. Uterus extends anteriorly into posterior third of forebody. Testes approximately equal, smooth to slightly irregular, situated side by side in hindbody. Bursa copulatrix well-developed; ejaculatory pouch absent; prostatic glands present.

Type Species: *Procyotrema marsupiformis* n. sp.

Procyotrema marsupiformis n. sp.
(Figs. 1–9)

Description: (Measurements in mm, obtained from 25 specimens). Body large, elongated, 9.32–16.26 in length. Forebody (5.74–10.27 × 1.22–2.24) approximately twice the length of, and slightly narrower than the hindbody (3.48–5.99 × 1.50–2.49), usually with a slight constriction at the anterior third. Anterior and posterior ends spatulate, but greater part of worm approximately cylindrical. Pseudosuckers, with weakly developed glands, at level of pharynx. Oral sucker (0.210–0.462 × 0.168–0.441) terminal, weakly developed. Ventral sucker (0.126–0.231 × 0.105–0.251) smaller than oral sucker, not raised above body surface, just anterior to holdfast organ averaging 1.16 from anterior end of oral sucker. Holdfast well developed, extending from ventral sucker to posterior end of forebody, contained within a pouch formed by the ventral wall of forebody; protrudes slightly beyond pouch; anterior border 0.932–1.66 from anterior end; surface directed ventrally with longitudinal slit-like cavity, latter more evident in posterior half; holdfast glands well distributed. Prepharynx absent, pharynx (0.357–0.441 × 0.336–0.441) well developed; esophagus apparently absent; ceca extend posteriorly to just behind testes. Vitellaria follicular, restricted to posterior 2/3 of forebody, in ventral pouch, holdfast and body proper; in 2 lateral fields separated by very narrow, median, vitelline-free area.

Testes 2, large, elongate-ovoid, smooth to irregular in contour, situated side by side in the hindbody, rather close together but not contiguous. Left testis (1.96–4.00 × 0.504–0.840) slightly longer than the right one (1.93–3.92 × 0.504–0.840). Each vas efferens arises from medial sur-

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* Supported in part by the North Carolina State College Faculty Research and Development Fund.

face of respective testis at anterior third, proceeding anteriorly and emptying separately into highly convoluted seminal reservoir. Latter at level of and dorsal to vitelline reservoir, between testes; vas deferens arises from ventro-posterior surface of seminal reservoir proceeding posteriorly between testes as a closely looped tube to seminal vesicle at posterior level of testes. The slightly muscular ejaculatory duct proceeds posteriorly from seminal vesicle to dorsal genital atrium, does not join uterus. Prostatic glands present along proximal two-thirds of duct. Ejaculatory pouch absent.

Ovary (0.200–0.462 × 0.570–0.693) transversely elongated, situated dorsally in anterior part of hindbody. Oviduct arises from dorso-posterior surface of ovary, extending posteriad, receiving common vitelline duct just before entrance into anterior right side of large Mehlis' gland. Laurer's canal present. Vitelline ducts extend posterio-mesad to posterio-ventral surface of vitelline reservoir; latter directed anterio-dorsad from level of seminal reservoir to common duct. Ootype in posterior portion of Mehlis' gland. Ascending limb of uterus arises from posterior surface of Mehlis' gland, proceeding anteriorly past ventral surface of ovary as a convoluted tube into posterior third of forebody and holdfast. Descending limb passes posteriad in midventral plane, curving dorsally in posterior region of hindbody to empty into genital atrium separate from and ventral to ejaculatory duct. Eggs (0.105–0.126 × 0.063–0.084) numerous, with a small operculum. Excretory pore terminal.

Host: Procyon lotor lotor (Linnaeus).

Habitat: Pancreatic duct.

Locality: Wake County, Raleigh and Pasquotank County, North Carolina.

Specimens: U.S.N.M. Helm. Coll. No. 38372, type, paratype, sagittal sections.

Superficially this trematode resembles *Duboisiella proloba* Baer, 1938. However, the structure of the holdfast is characteristic of the Diplostomatines Dubois, 1936. *Procyotrema* differs from *Duboisiella* in the structure of the holdfast, the presence of pseudosuckers, arrangement of testes, absence of ejaculatory pouch, and the anterior position of the uterus, which in *Duboisiella* is restricted to the ventral pouch of the forebody. It shows similarities to *Pharyngostomum* Ciurea, 1922 and *Pharyngostomoides* Harkema, 1942 in the arrangement of the testes. However, its large size, structure of the holdfast, and the presence of the ventral pouch easily differentiate it from these genera.

Dr. George R. La Rue, who kindly examined the specimens, is of the opinion that the ventral pouch may be the result of convergence of the lateral folds of the forebody so characteristic of the Diplostomatidae. Appreciation is expressed to Dr. La Rue and to Mr. Allen McIntosh for their criticisms and suggestions.

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Explanation of Figures

Abbreviations: AUT, ascending uterus; C, caecum; CB, copulatory bursa; DEJ, ejaculatory duct; DUT, descending uterus; GP, genital pore; HFC, holdfast cavity; HFO, holdfast organ; HFS, holdfast slit; HT, host tissue; LC, Laurer's canal; MG, Mehlis' gland; OT, ootype; OV, ovary; PH, pharynx; PSS, pseudosucker; T, testis; UT, uterus; VD, vas deferens; VE, vas efferens; VGL, vitelline gland; VR, vitelline reservoir. (Scales in mm.)

Figure 1. Whole mount, ventral view.

Figure 2. Sagittal section.

Figure 3. Cross section through pseudosuckers.

Figure 4. Cross section through anterior region of holdfast.

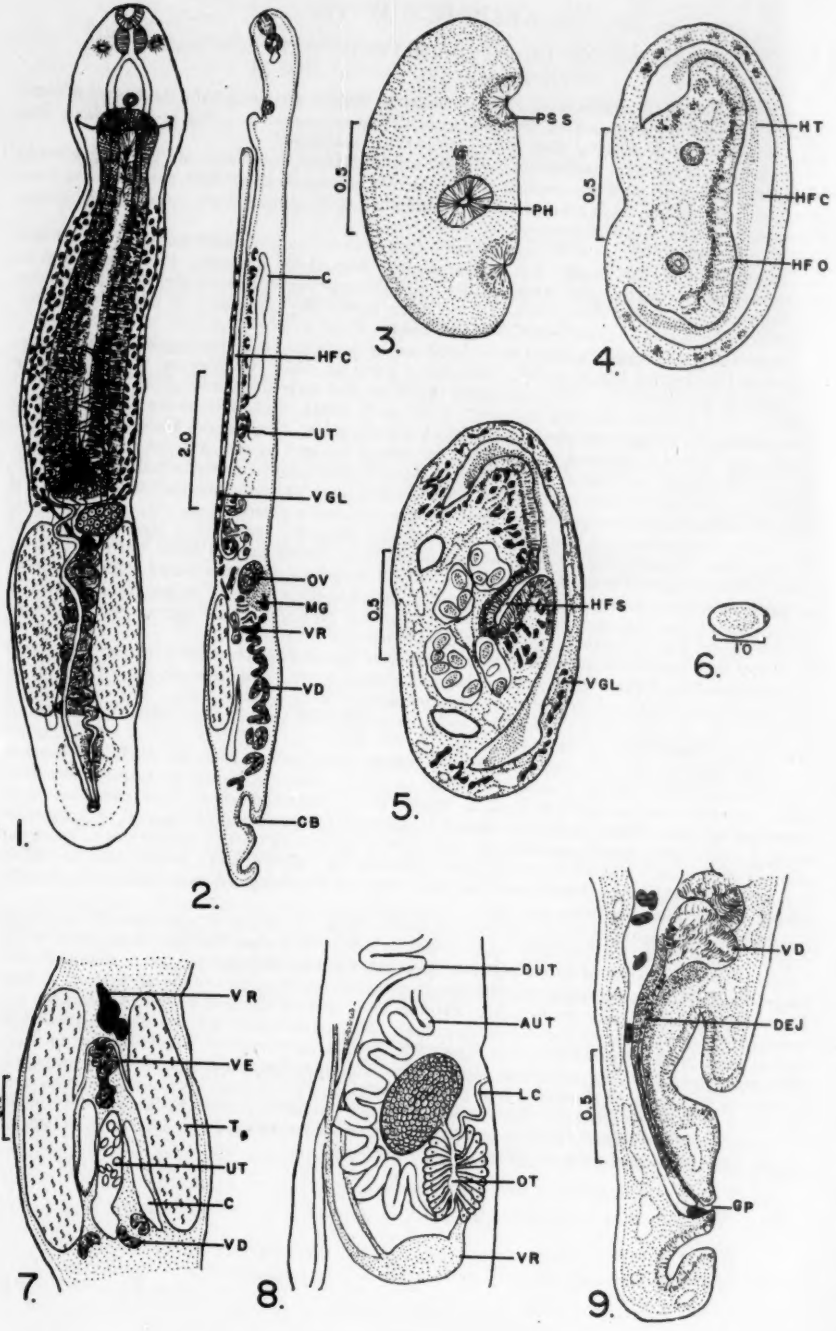
Figure 5. Cross section through posterior region of holdfast.

Figure 6. Operculated egg. Scale = 0.1 mm.

Figure 7. Frontal section, level of testes.

Figure 8. Graphic reconstruction of ovarian complex.

Figure 9. Sagittal section, posterior end, VD = Seminal vesicle.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RESEARCH NOTE

A PROCEDURE FOR DELAYED COLLECTING OF ECTOPARASITES FROM SMALL CAPTURED HOSTS

Parasitologists frequently have an opportunity to collect a considerable number of animals without having the time to search them immediately for ectoparasites. This is particularly true if a quantitative study is being made of the ectoparasite population.

If ice or refrigerators are handy, the study can be delayed, but frequently not long enough. Sometimes it is desirable to accumulate a large number of animals in summer, then examine them in winter. Sometimes, as in my case, it is desirable to collect animals while on a trip and examine them at odd hours in a laboratory.

When collecting chiggers from hosts for a quantitative study in New Guinea, I found that the host's bodies did not become too unpleasant in 2 days if the intestines were first removed. However, other duties too often interfered with collecting within that short period, and removing intestines often took too much time and too often fouled the parasites. A neater procedure, adaptable to irregular working periods, was necessary.

Bodies of the animals therefore were enbalmed by injection of formaldehyde into abdominal and pleural cavity and lightly enfolded in several turns of cloth. Commercial formaldehyde in full strength is satisfactory. The more used, the faster and surer the preservation, but care must be taken while injecting to avoid leakage from the body which might kill nearby parasites. Uninfested portions of the body should be selected for the point of injection. Ears, anal area and nasal cavity should be avoided, because they are usually infested by ectoparasites. In animals as large as bandicoots and rabbits, 40 cc of formaldehyde into chest and abdominal cavities is desirable. In the tropics such animals also should receive an injection into the larger muscles if they are to be kept longer than 4 or 5 days. For mice 5 ml is enough.

Tails of mammals and birds may be cut off and discarded unless it is known or believed that they bear ectoparasites. Ears of mammals and birds having ears so large that they may be closed when the animal is enfolded should be cut off and saved with the body; otherwise, chiggers that would detach and come out may be imprisoned in the ear cavity or become imbedded in wax. When being removed, the ears should be handled carefully with a forceps to avoid damaging chiggers that are attached to the surface or margin.

After injection, the host body should be wrapped lightly in cloth of a mesh that will retain the ectoparasites. (Black cloth is desirable; ectoparasites are easily seen on it.) The ends of the cloth should be closed by a double fold and secured with cord or wire. The hosts should then be dried in a cool, airy place and protected against mold and museum pests. If not killed, mesostigmatid mites may continue breeding.

When time is available, the ectoparasites may be hand picked from the cloth and brushed from the dried body which is easily examined in detail. Account should be taken of the individual parasites that are already dead or unable to extricate themselves from ear wax, nasal secretions or scabs when the host is captured. Generally they should be counted as a separate group in quantitative studies, particularly those having to do with tests of insecticides or changing weather conditions. I based my study (Am. Midland Nat. **55**: 382–392) on all, dead and alive, that were present in the external ears, but freed them by dissolving the wax in alcohol and light oil and teasing them free with a needle.

Various devices other than combing and hand picking are helpful in collecting the parasites. Buehler (1951, CDC Bull. **10**: 24) recommended a tube with a claw-like device, attached to the windshield-wiper system of an automobile. The claws passing through the fur of mammals dislodged many of the ectoparasites which were then sucked into a receptacle by the exhaust system. Lipovsky (1951, Jour. Kansas Ent. Soc. **24**: 151–156), washed the host bodies in detergent and removed ectoparasites from the supernatant fluid. This caused most but not all ectoparasites to release their hold. Cook (1954, Ent. News **61**: 35–37), recommended a modification of Hopkins' technique for freeing arthropod parasites that are imbedded in the skin or under scabs. It consists of cutting dried skins into 1 or 2-inch squares, boiling them in a chemical solution until the skin and hair are dissolved, then straining out the parasites. If the Cook procedure would be satisfactory for animals that have been treated with formalin, I do not know.—CARL O. MOHR, *Illinois Natural History Survey, Urbana, Illinois.*

OBSERVATIONS ON THE MORPHOLOGY AND LIFE CYCLE OF *STRIGEA ELEGANS* CHANDLER & RAUSCH, 1947 (TREMATODA: STRIGEIDAE)*

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In May, 1954, a live snowy owl, *Nyctea nyctea* L., was given to the writer by the Ontario Department of Lands and Forests. This owl is normally an inhabitant of the tundra, but had apparently spent the winter in southern Ontario where it was captured. At autopsy, 63 *Strigea elegans* and numerous examples of a species of *Neodiplostomum* were found. The species of *Neodiplostomum* will be described elsewhere.

At this time the writer was studying the life cycles of two species of *Alaria,* and was interested in the phylogeny of the curious life cycles of *Alaria* spp., especially the position of the mesocercaria. When it was recalled that Lutz (1929, 1933a) had described a mesocercarial stage for *Strigea vaginata,* it was considered pertinent to a study of phylogeny to work out the life cycle of *Strigea elegans.* This has been done roughly and some of the stages have been studied. It was found that a freshwater snail, *Gyraulus parvus* (Say), could act as first intermediate host; that cercariae penetrated tadpoles of *Rana sylvatica, R. clamitans,* and *Bufo americanus* and developed into mesocercariae; that mesocercariae developed into tetracotyles in garter snakes (*Thamnophis sirtalis*), domestic ducks (*Anas boschas*), and apparently, great horned owls (*Bubo virginianus*). It appears that mesocercariae cannot become adults in the definitive host and that 4 hosts are required in the life cycle.

HISTORY

Strigea elegans was described by Chandler & Rausch (1947) from the great horned owl, *Bubo virginianus,* taken in Wisconsin, U.S.A. It was redescribed by Dubois & Rausch (1950) from the same host and locality.

Partial life cycles are known for 2 species of the genus *Strigea.* Braun (1894) reported that tetracotyles, *Tetracotyle colubri* von Linstow, from the snake, *Tropidonotus natrix* L., developed into *Holostomum variabile* Nitzsch in the owl, *Strix aluco.* Dubois (1938) considered this species to be either *Strigea strigis* or *Strigea falconis.*

The life cycle of *Strigea vaginata* (Brandes) was worked out by Lutz (1929, 1933a). To clarify Lutz's contribution, his results will be considered in some detail. In a preliminary report, Lutz (1929) made the following observations:—1. Two kinds of mesocercariae occurred naturally in frogs. 2. A furcocercaria from the snail, *Planorbis anatinus,* developed into 1 of the 2 kinds of mesocercariae in frogs. 3. One of the 2 kinds of mesocercariae developed into a tetracotyle when fed to a pigeon and a duckling. 4. Tetracotyles in naturally infected freshwater fish, snakes

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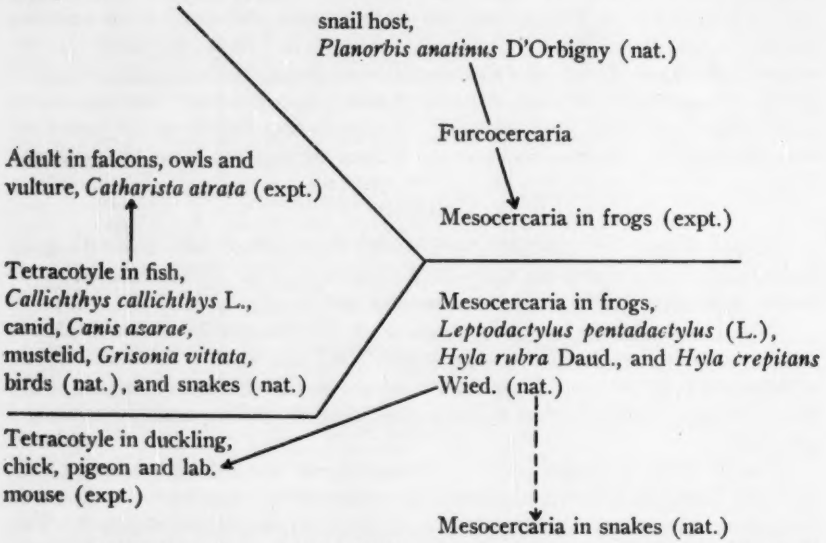
and mammals became *Holostomum vaginatum* (= *Strigea vaginata*) when fed to birds of prey. 5. Tetracotyles in naturally infected birds became *Strigea falconis brasiliana* in birds of prey.

On the basis of these observations, Lutz stated that these forms have a life cycle involving 4 hosts. In a fuller account, Lutz (1933a) added further observations on the life cycle of *Strigea vaginata*. He states that he (1929) fed mesocercariae from frogs to birds and obtained a tetracotyle like the one which he found in naturally infected birds. He states here (1933a) that tetracotyles in naturally infected birds became *Strigea vaginata* when fed to birds of prey, whereas he had said earlier (1929) that tetracotyles in birds were the larvae of *Strigea falconis brasiliana* and that tetracotyles in fish, snakes and mammals were the larvae of *Strigea vaginata*. Apparently, Lutz considers his identification of the adults which developed from tetracotyles in birds to have been incorrect in 1929, since he does not refer to *Strigea falconis brasiliana* in his later paper (1933a) but states that all of the stages belong to *Strigea vaginata*. The life cycle of *Strigea vaginata*, as worked out by Lutz, is given below. The solid arrows represent experimental demonstrations, and the dotted arrow represents the course suggested by Lutz. The abbreviation (nat.) after the name of a host means a natural infection, and the abbreviation (expt.) means an experimental infection. The solid lines indicate the steps demonstrated experimentally. That is, there were no experimental links among the 3 parts of the life cycle.


snail host,
Planorbis anatinus D'Orbigny (nat.)
Furcocercaria
Mesocercaria in frogs (expt.)
Adult in falcons, owls and vulture, *Catharista atrata* (expt.)
Tetracotyle in fish, *Callichthys callichthys* L., canid, *Canis azarae*, mustelid, *Grisonia vittata*, birds (nat.), and snakes (nat.)
Mesocercaria in frogs, *Leptodactylus pentadactylus* (L.), *Hyla rubra* Daud., and *Hyla crepitans* Wied. (nat.)
Tetracotyle in duckling, chick, pigeon and lab. mouse (expt.)
Mesocercaria in snakes (nat.)

Lutz (1933a) does not mention finding the cercaria of *S. vaginata*, although it would appear from his paper in 1929 that he found a furcocercaria in *Planorbis anatinus* which he considered to be the cercaria of *S. vaginata*. He does not figure or describe the cercaria or mesocercaria here (1929), but in his subsequent paper (1933a) figures and describes the mesocercaria, referring to it as "Pseudodistomula mit acht Drüsenzellen." Although Lutz did not describe or figure the cercaria, a comparison of this mesocercaria with known strigeid cercariae suggests that it is an

enlarged cercarial body, possessing no metacercarial or adult characters, and that it is comparable with, although morphologically dissimilar to, the mesocercaria of *Alaria*.

MATERIALS AND METHODS

Adult worms recovered alive from the snowy owl were fixed by dropping them into boiling 10% formalin. Whole mounts were stained with Mayer's haemalum, cleared in methyl benzoate, and mounted in Canada balsam.

Methods used for collecting and culturing eggs, exposing snails to miracidia, observing the escape of cercariae, and fixing the various stages were the same as those of Pearson (1956).

Snails used in experimental exposures were raised from egg masses laid in the laboratory. Tadpoles of *Rana clamitans* were raised in the laboratory from egg masses. Two groups of tadpoles collected from a temporary pond outside Toronto were identified respectively as *Bufo americanus* by their small size and median anus, and as *Rana sylvatica* by their dextral anus, sinistral spiracle, infolded lips, and tooth rows and 3/4 and 4/4 (see Orton, 1952). As a control, 42 tadpoles of *Rana sylvatica* were dissected 9 to 20 days after collection (to allow recently entered parasites to develop) and 50 tadpoles of *Bufo americanus* were dissected 27 days after collection. No strigeid parasites were found in any of these tadpoles. Garter snakes, *Thamnophis sirtalis* (L.), born in the laboratory, were reared on earthworms and marine fish. A control group of 15 snakes was examined and found free of infection with strigeids. Ducklings, *Anas boschas*, were obtained from a hatchery. Two fledgeling great horned owls, *Bubo virginianus* (Gmelin), were held in the laboratory for 1 month before being fed experimentally. During this month, no eggs of *Strigea elegans* were found in numerous fecal samples from the owls.

LIFE CYCLE

Large numbers of eggs of *Strigea elegans* and *Neodiplostomum* sp. were recovered from the intestinal contents of the snowy owl. This mixture of the eggs of 2 species was used in the experimental infection of snails. Two types of cercariae were found in these snails. The cercaria from *Gyraulus parvus* developed into a mesocercaria in tadpoles and later into a tetracotyle in birds; this one must have belonged to *Strigea elegans*. The other cercaria from *Physa* sp. developed into a diplostomulum in tadpoles and must have belonged to *Neodiplostomum* sp.

The eggs in this mixture were in early stages of cleavage when first taken from the intestines. Miracidia developed in about 11 days at room temperature, but at 8° C in darkness many eggs were not fully developed in 11 months. These chilled eggs rapidly completed development when returned to room temperature, and infective miracidia of both species hatched out of many of them.

Laboratory-reared snails of various ages were exposed to infection; the species and numbers of each exposed were as follows: 6 *Planorbula armigera* (Say), 4 *Helisoma trivolvis* (Say), 4 *Helisoma antrosa* (Say), 45 *Gyraulus parvus* (Say), 31 *Physa* sp., and 6 *Lymnaea palustris* (Müller). Cercaria-producing infections developed in 6 *Gyraulus parvus*, cercariae emerging first 26 days after exposure.

Tadpoles of *Rana sylvatica* LeConte, *Rana clamitans* Latreille, and *Bufo americanus* (Holbrook) were exposed to cercariae. Cercariae penetrated all 3 species of tadpole and developed into mesocercariae in about 2 weeks at summer temperatures in *Rana sylvatica* and *Bufo americanus*. None of the tadpoles of *Rana clamitans* lived for more than 13 days after exposure to cercariae, and while 2 tadpoles were negative 9 days after exposure, 1 tadpole was infected 13 days after exposure with mesocercariae which were almost fully developed. It appears from this that mesocercariae can develop in tadpoles of *Rana clamitans*, but that they may not be as suitable as tadpoles of the other species.

Mesocercariae from tadpoles were fed to 1 garter snake, 3 domestic ducklings, and 2 young great horned owls. The garter snake was examined 101 days later,

when encysted, encapsulated tetracotyles were found on and less commonly within, the intercostal muscles, on both external and coelomic faces, over most of the length of the body. The ducklings were examined 22, 23, and 64 days after feeding; in them encapsulated, encysted tetracotyles, all apparently fully developed, were found in the fascia over the muscles of the neck, esophagus, crop, and trachea, and less commonly over the breast, on the lateral face of the thighs, and over the abdomen. No tetracotyles were found in the body cavity.

Tetracotyles from these ducklings were fed to 4 garter snakes which were examined 1, 8, 30 and 68 days later. No tetracotyles were found in these snakes. That is, tetracotyles failed to re-establish themselves in another host, and so appear to lack the ability to re-establish, which was found in the diplostomulum of *Pharyngostomum cordatum* by Wallace (1939) and in the mesocercariae of *Alaria* spp. (see Pearson, 1956).

It was assumed that tetracotyles from the garter snake and ducklings would develop into adults in the definitive host, and so mesocercariae were fed to the 2 young great horned owls to see whether the life cycle could be completed in 3 hosts as in *Alaria* spp. Following the feedings, feces from the owls were examined at intervals until the birds were killed and examined 21 and 113 days later. No eggs of *Strigea elegans* were found in the feces and no worms were found in the intestines. However, encapsulated, encysted tetracotyles were found in the loose fascia over the skin, muscles, esophagus, and trachea in the neck.

It appears that 21 days is the maximum time for the development of the tetracotyle, as only fully developed tetracotyles were found in the duck and the owl examined at this time. This development in warm-blooded hosts is more rapid than that of tetracotyles developing in cold-blooded hosts. For example, Mathias (1925) found that the tetracotyle of *Strigea tarda* (synonym of *Cotylurus brevis* Dubois & Rausch, according to Dubois, 1953) required 6 weeks to develop in the snail, *Lymnaea stagnalis*. Olivier (1940) found that tetracotyles of *Apharyngostrigea pipientis* were immature at 21 days in tadpoles and underwent considerable development after this time.

These findings suggest that mesocercariae of *Strigea elegans*, unlike those of *Alaria* spp., cannot attain the adult state in the definitive host, but must first become a tetracotyle in a third intermediate host. From this it appears that *Strigea elegans* has an obligatorily 4-host life cycle.

The life cycle of *Strigea elegans* is similar to that suggested and partially demonstrated by Lutz (1929, 1933a) for *Strigea vaginata*. Lutz found natural infections with tetracotyles in a wide variety of hosts including amphibians, fish, snakes, birds, and mammals. Tetracotyles from all of these hosts were said to have developed into *Strigea vaginata* when fed to hawks and vultures. The occurrence of tetracotyles in amphibians is surprising, since neither Lutz nor the writer found any evidence that mesocercariae developed further in the amphibian host. It should be pointed out that neither Lutz nor the writer fed mesocercariae to frogs to see whether they would re-establish themselves as mesocercariae or develop into tetracotyles.

DESCRIPTION OF STAGES

Adult (Figs. 1–3)

The description is based on the examination and measurement of 13 whole mounts, and the examination of serial sagittal sections of three worms, and mounts

of the ventral half of the forebody of 2 worms. Unless otherwise stated measurements are in microns.

Diagnosis: Total length 2.7 (2.1–3.3) mm, body curved dorsally. Forebody bulbiform; length 750 (600–970), width (for two specimens) 620 and 700, thickness 740 (600–890); with dorsal margin distinctly in advance of ventral margin; dorsal margin trilobed, with oral sucker in median lobe. Hindbody separated from forebody by constriction ventrally and laterally but not dorsally; in shape a long ellipse; length 1.9 (1.5–2.5) mm, width 565 and 595, thickness 750 (570–850); ratio hindbody-forebody 2.6 (2.2–3.2).

Oral sucker terminal; length 130 (90–150), width 120 (100–150). Pharynx contiguous with oral sucker; length 100 (90–140), width 100 (70–130), almost equal to oral sucker. Ventral sucker large, with mouth directed anteriorly; length 190 (160–200), width 170 (140–200). Holdfast of 2 lobes; dorsal lobe larger and having at its base U-shaped holdfast gland 120 (80–150) in length, 130 and 140 in width and 360 (270–430) in thickness; pair of lateral sucking cups prominent; in form of semi-lunar to rectangular pit, lying laterally just behind pharynx (Fig. 2).

Ovary transversely elongate; length 160 (110–230), width 190, 220, thickness 200 (170–260); median and situated well back in hindbody at 26 (21–35)/100 of hindbody length. Anterior testis dumbbell-shaped and irregularly lobulated; length 290 (160–360), width 340 and 390, thickness 420 (380–520). Posterior testis with 2 elongate lateral lobes joined by an anterodorsal bridge; irregularly lobulated; length 380 (260–500), width 320 and 390, thickness 440 (360–550). Vitelline follicles small and irregular; in forebody, densest near junction of forebody and hindbody, extending forward dorsally to, or almost to, pharynx, laterally about half way to anterior end, and present ventrally typically near junction but sometimes extending as much as half way to anterior end; follicles extending into proximal half of holdfast lobes. In hindbody, vitelline follicles extending back dorsally and laterally to ovary or anterior testis, and ventrally as a broad band to, or slightly beyond, anterior margin of copulatory bursa. Vitelline reservoir and Mehlis' gland median, intertesticular. Ascending loop of uterus extending anterior to ovary for distance equal to length of ovary. Eggs 112–128 long by 71–77 wide. Vas deferens with loops anterior to anterior testis; seminal vesicle lying between and behind lateral lobes of posterior testis, length 220 (170–260) and thickness 240 (200–300); ejaculatory duct short, without trace of periprostate, joining dorsal wall of uterus within genital cone to form short hermaphroditic duct (Fig. 3). Copulatory bursa conspicuous; length 200 (140–270), width 300 (200–430); bursal region delimited externally by roughly parallel sides which contrast markedly with the tapering contour anterior to the bursa. Genital cone small, poorly delimited, variable in shape and degree of extension; length 130 (90–190), width 90 (80–100); with hermaphroditic canal lying axially and opening on the summit of the cone. "Ringnapf" small, strongly developed. Excretory pore dorsal, median, and at level of posterior margin of "Ringnapf".

Outer surface of forebody cup covered with fine spines; inner surface of cup spineless. Holdfast lobes with numerous large spines on free ends and on distal half of opposing faces. Hindbody spineless.

Host: Nyctea nyctea L.

Location: small intestine.

Locality: southern Ontario, May, 1954.

Specimens: U. S. Nat. Mus. Helm. Coll. No. 38381, also coll. Dubois, coll. Chandler, coll. author.

Comparisons. The material described here from the snowy owl differs in the following respects from that described by Chandler & Rausch (1947) and Dubois & Rausch (1950); (1) the average total length is greater, although the ranges overlap, 2.7 (2.1–3.3) mm as opposed to 1.55–2.45 mm and 1.77–2.31 mm (these measurements are those of Chandler & Rausch and of Dubois & Rausch in that order); (2) the ratio of the hindbody to the forebody is greater, 2.6 (2.2–3.2) as opposed to 1.8 (1.4–2.3) given by Dubois & Rausch, but again the ranges meet; (3) the genital cone is smaller, 130 (90–190) by 90 (80–100) microns as opposed to 170–220 × 145–200 microns given by Dubois & Rausch; (4) bursal region not delimited by a constriction as figured by Chandler & Rausch and described by Dubois & Rausch; (5) vitelline follicles typically do not extend as far forward on the ventral side of the forebody as figured by Chandler & Rausch and Dubois & Rausch.

These differences are not considered significant; nevertheless, in view of them, a description was thought to be warranted, especially as this paper is primarily concerned with the life cycle and larval stages and it was deemed essential that the species involved should be determined accurately.

Cercaria (Figs. 4–7)

The diagnosis and description are based on a study of living material; measurements (average and range) in microns are given for 15 fixed specimens.

Diagnosis: Furcocercous, longifurcous, pharyngeate, not ocellate. Body elongate; length 135 (120–160) and width 38 (30–50), with 1 pair of triple, and 2 pairs of single hairs or flagellets laterally, the triple pair being at junction of oral sucker and body and single pairs being one just behind this junction and other at level of ceca. Oral sucker large, with subterminal opening; 38 (34–43) long and 29 (24–33) wide; functioning like anterior organ of schistosome cercariae. Prepharynx short, 3 (2–4). Pharynx small, 10 (9–11) long and 11 (10–13) wide. Esophagus long. Ventral sucker smaller than oral, 21 (17–23) long and 25 (22–30) wide; with anterior margin 80 (40–95) from anterior end. Bifurcation of gut immediately anterior to ventral sucker; ceca permanently constricted in 3 places, extending almost to excretory bladder. Unpigmented eyespots absent. Four pairs of coarsely granular, post-acetabular penetration glands, in linear series dorsal to ceca; ducts of penetration glands arising antero-medially and lying ventral to duct from cell behind, ducts enlarged within oral sucker, opening separately at sides of mouth. Genital rudiment small, undifferentiated, lying between ends of ceca.

Tailstem with parallel sides, with about 30 annular constrictions, and bearing many long, slender hairs (flagellets), length equal to width of tailstem; length 130 (120–140) and width 30 (21–35). Furcae without finfolds; length 140 (125–150), width (dorso-ventral) 24 (21–25); with small projection (2 hairs ?) at tip; flagellets not seen.

Flame cell formula 2 [(1+1) + (1+1+ (1))]; bladder bicornuate; bladder arms convoluted at level of posterior margin of ventral sucker, without cross connection, anterior blind tubule, or tufts of cilia (Fig. 4); flame cells in tailstem at base, asymmetric; excretory atrium present between body and tailstem; caudal excretory tubule surrounded by elongate, fusiform cells without processes to tailstem wall (caudal bodies ?); furcal excretory tubules opening mid-furcally, and dorsally.

Circum-oral area spineless, without group of forwardly directed spines; oral hood a dense band of 9–12 rows of spines extending back to middle of oral sucker. Ventral sucker with 3 staggered rows of small spines. Spines on body denser anteriorly, covering whole dorsal surface (Fig. 4), ventral surface with 2 large spineless areas one in front and one behind ventral sucker, and with denser patches of spines postero-lateral to ventral sucker (Fig. 5). Tailstem with 4 rows of spines, 2 dorso-lateral and 2 ventro-lateral, continuing onto dorsal and ventral margins of furcae.

Host: Gyraulus parvus (Say) (experimental).

The oral sucker functions as a penetrating organ and not as a sucker, and is perhaps best described as an anterior organ (Miller, 1926). There are 2 distinct changes during the action of this organ. First, the oral opening is retracted so that the tip of the organ bearing the oral hood is invaginated (Fig. 6b), and then the whole organ is partially retracted within the body so that an invagination is formed at the junction of the cuticle over the anterior organ with that of the body (Fig. 6c and d).

The tail stem is similar to that described by Pearson (1956) for the cercariae of *Alaria canis* and *A. arisaemoides,* in having an annulated cuticle with circular muscle fibers internal to the grooves in the cuticle, 2 main groups (dorsal and ventral) of longitudinal bipolar muscle cells with striated processes, and a caudal excretory tubule running through the hollow, fluid-filled central part. The caudal excretory tubule is surrounded throughout its length by elongate, fusiform cells which are not connected to the wall of the tailstem (Fig. 5). Such cells as these have been called caudal glands (Dubois, 1929; Miller, 1926) and caudal bodies (many authors, e.g. Wesenberg-Lund, 1934; Cort & Brooks, 1928), whereas cells about the caudal excre-

tory tubule which have processes running to the tailstem wall have been called stellate cells (Olivier & Cort, 1941) and caudal supporting cells (Pearson, 1956). The observation of Olivier (1942, Pl. II, Figs. 4 & 5) that both types of cell are present in the tailstem of *Cercaria elodes* strongly suggests that these two types of cells are modifications of a single cell type. It also appears that these cells can be considered as supporting or strengthening the thin-walled caudal excretory tubule whether they are connected with the tailstem wall or not.

Escape of Cercariae. To observe the route and method of escape of cercariae a snail was held under the dissecting microscope, using the method described by Pearson (1956). Cercariae, which had escaped from daughter sporocysts lying in the peri-hepatic haemocoele, made their way to the ring vessel of the collar, presumably by way of the peri-rectal blood sinus. Cercariae crawled and swam along the ring vessel, and then entered a radial branch running to the free edge of the collar. At a point where this radial vessel is near the surface of the body, cercariae escaped through the wall of the blood vessel and the overlying epidermis in the following manner: The body of the cercaria wormed its way through the tissues to the outside and then remained quiescent while the tailstem and the bases of the furcae were slowly extruded, evidently under pressure. Then, with a few flicks of the tail, the tips of the furcae were freed, and the cercaria swam away. Almost all of the cercariae observed escaped through the same point in the snail's integument. From this it appears that escaping cercariae create, and maintain by frequent passage, a permanent wound in the snail's epidermis. This permanent wound, or escape pore, is on the top of a small epidermal papilla, and is closed by contraction of the epidermis following the escape of each cercaria.

These observations agree closely with those of Pearson (1956) on cercariae of *Alaria canis* and *A. arisaemoides* both as to route and manner of escape and to the presence of fixed escape pores. The writer (unpublished) has observed a schistosome cercaria of the Ocellata group (*cf. Cercaria pseudocellata* Szidat & Wigand, 1934) escaping from the collar of the snail, *Lymnaea stagnalis,* in the same manner through fixed escape pores. These observations are not in accord with those of Duke (1952) who observed that cercariae of *Schistosoma mansoni* escaped by the rupture of vesicles formed about cercariae in the integument of the snail, *Australorbis glabratus.*

After escaping from the snail, cercariae hang body downwards in the water (Fig. 7c) and occasionally swim upwards a short distance (Fig. 7b). When cercariae stopped swimming, the angle between furca and tailstem, which was acute during swimming (Fig. 7a) suddenly increased to nearly 180° (Fig. 7b), and then returned to slightly greater than 90°, the resting angle (Fig. 7c). Cercariae were evenly distributed in the container.

Related Cercariae. The most closely related cercaria is probably that which Lutz (1929) found in *Planorbis anatinus* and which became a mesocercaria with 4 pairs of post-acetabular penetration glands in tadpoles. Lutz considered that this cercaria, which he did not describe, belonged to *Strigea vaginata,* although he did not demonstrate this experimentally. Dubois (1938), describing the life cycle of *Strigea vaginata,* stated incorrectly that the cercaria is *Dicranocercaria gyrinipeta* Lutz, 1934. Actually, Lutz (1933b, not 1934) did not connect this cercaria with an adult; but from the description of the cercaria and the observation that it develops

in tadpoles into a mesocercaria (Pseudodistomulum of Lutz) with 2 pairs of pre-acetabular penetration glands, it is highly probable that *D. gyrinipeta* is the cercaria of a species of *Alaria*.

Of the described species of pharyngeate, distomate, longifurcous furcocercariae with 4 pairs of post- or para-acetabular penetration glands, *Cercaria rufula* Fain, 1953 most closely resembles the cercaria of *Strigea elegans* (*cf.* Fain, 1953). These two cercariae have the following features in common: Development in a pulmonate snail; pre-oral spines absent; caudal bodies fusiform, without processes; and flame cell formula $2[(1+1)+(1+1+(1))]$, with no commissures or blind tubules, with excretory pore mid-furcal. However, *C. rufula* differs in having fewer rows of spines in the oral hood, unpigmented eyespots, and finely granular penetration glands, the last 2 of which (on each side) are opposite rather than tandem.

The remaining related cercariae may be divided into 2 groups. The first group is characterized by the flame cell formula $2[(1+1)+(2+2+(1))]$ and the presence of 1 or more commissures between the bladder arms, features which clearly distinguish the members of this group from cercaria of *Strigea elegans*. This first group contains the cercariae of *Apatemon gracilis* described by Szidat (1931), *A. globiceps* [= *Cercaria burti* Miller, 1923, redescribed by Cort & Brooks, 1928; = *Cercaria pseudoburti* Rankin, 1939; see Stunkard et al, 1941, and Olivier, 1942 for relationships and proposed synonyms]; *A. intermedius* [= *Cercaria lessoni* Johnston & Beckwith, 1947]; *Cercaria pygocytophora* Brown, 1931 (*cf.* Brown, 1931); *Cercaria longiremis* Wesenberg-Lund, 1934, which may be the same as the cercaria of *A. gracilis; Cercaria helvetica* XXXI Dubois, 1929 (the form described by Wesenberg-Lund, 1934, under this name differs in the arrangement of the penetration glands and in having a single bladder commissure); *Cercaria wansoni* Fain, 1953 (*cf.* Fain, 1953); *Cercaria hamburgensis* Komiya, 1938 (*cf.* Komiya, 1938); and *Cercaria okobojensis* Brooks, 1943 (*cf.* Brooks, 1943). *Dicranocercaria bdellocystis* Lutz, 1933 (*cf.* Lutz, 1933b) may belong in this group, although it is impossible to tell from the description, as it developed into a tetracotyle in leeches which in turn developed into an immature *Apatemon* sp.

The second group of cercariae is an unnatural assemblage of species of unknown affinities, and includes: *Cercaria caperata* Olivier, 1942, which differs in having 2 pairs of flame cells in the tail stem and an incomplete bladder commissure; *Cercaria stephensi* Brooks, 1943, which differs in having pre-oral spines, unpigmented eyespots, para-acetabular penetration glands, and 2 flame cells in the tailstem; *Cercaria angelae* Johnston & Simpson, 1944, which differs in having 3 groups of flame cells on the anterior collecting tubule, the excretory pores terminal on the furcae, pre-oral spines, and in developing into a tetracotyle in tadpoles; and *Cercaria gracillima* Faust, 1917, an inadequately described species which differs in snail host and in having unpigmented eyespots (*cf.* Faust, 1917).

Johnston & Simpson (1944) suggested that *Cercaria angelae* is probably the cercaria of a species of *Strigea* or of *Apharyngostrigea;* this seems most unlikely considering the differences between this cercaria and that of *S. elegans* given above, and the differences in excretory system and tailstem between it and the cercaria of *Apharyngostrigea pipientis* described by Olivier (1940).

Mesocercaria (Figs. 8–10)

The diagnosis and description are based on the study of living and fixed material; measurements (average and range) in microns are given for 15 fixed specimens.

Diagnosis: Body oval, somewhat flattened dorso-ventrally; length 250 (220–290) and width 93 (71–110). Oral sucker large, terminal, with oral opening slightly subterminal; length 56 (50–65) and width 44 (41–50); invaginable and partially retractable, functioning as an anterior organ; no gland cells (head organ) seen within it. Prepharynx short, 6 (4–8) long. Pharynx small, globular; length 12 (10–14) and width 13 (12–14). Esophagus short, inflated; 9–13 long; separated from each caecum by a sphincter. Ceca inflated, thin-walled, sometimes septate, sinuous, extending almost to excretory bladder. Ventral sucker large and powerful, anterior margin situated 120 (100–140) from anterior end; length 38 (34–41) and width 41 (37–47). Four pairs post-acetabular penetration glands in 2 longitudinal rows dorsal to ceca; strongly lobed in contracted worm; contents of variable appearance, sometimes coarsely and sometimes finely granular; ducts arising antero-medially and running dorsal to bodies of penetration gland cells, with posterior ducts lateral and dorsal to more anterior ducts; ducts coiled 1½–2 times between penetration glands and oral sucker; nuclei of gland cells with large, central nucleolus and lying in a clear vesicle within granular cell; ducts swollen within oral sucker and opening separately in 2 groups antero-lateral to oral opening. Genital rudiment small, undifferentiated, lying between ends of ceca. Flame cell formula 2 $[\{(3+3+3)+(3+3+3)\}+\{(3+3+3)+(3+3+3)\}]=72$; with pre-, post-, and circum-acetabular commissures variously developed (Fig. 10); with terminal excretory pore, small bicornuate bladder, and with bladder arms convoluted behind ventral sucker, convolutions without tufts or patches of cilia; of each group of 3 triplets of flame cells arising from the collecting tubules 1 group lying ventrally and 2 groups dorsally; without posterior blind tubule on posterior collecting tubule (Fig. 9). Circumoral area spineless, without group of forwardly directed spines; oral hood of 9–12 rows of spines, encircling anterior half of oral sucker; ventral sucker with 2–4 staggered rows of spines; spination of body same as cercaria, with spineless area about excretory pore.

Hosts: Rana sylvatica LeConte (tadpole; experimental), and *Bufo americanus* (Holbrook) (tadpole; experimental). U. S. Nat. Mus. Helm. Coll. No. 38383 from toad tadpole.

The musculature of the body wall is in 3 layers; an outer, continuous circular layer beneath the cuticle, a middle layer of longitudinal anastomosing fibers, and an inner layer made up of 2 groups overlapping laterally, 1 dorsal and 1 ventral, of discrete diagonal fibers in triplets dorsally and in pairs ventrally, lying anterior to the ventral sucker (Fig. 8). This arrangement of muscle layers is similar to that described by Pearson (1956) for the mesocercaria of *Alaria arisaemoides*. A muscle with branched ends runs from the ventral sucker to the body wall on each side.

It is interesting to note that while the reserve excretory system is not developed at all in the cercaria (Fig. 4), the beginnings of this system are variably but often well developed in the mesocercaria (Fig. 9). A nucleus similar to the flame cell nuclei in staining reaction was seen associated with the tubules of each triad of flame cells. Similar nuclei similarly placed were observed by Pearson (1956, Figs. 21 and 30) in the young mother sporocyst and in the tail stem of the cercaria of *Alaria arisaemoides*.

Related Species. Only one other mesocercaria with 4 pairs of post-acetabular penetration glands has been described; this form is the "Pseudodistomulum mit acht Drüsenzellen" of Lutz (1933a). Lutz described and figured imperfectly this mesocercaria, apparently from material taken from a natural infection since he stated in the legend to the figure that the host was *Hyla crepitans* Wied.

It is surprising that mesocercariae of this type have not been found by other workers, especially when one considers the enormous number of frogs examined for parasites and recalls the abundance of reports on natural infections with mesocercariae of *Alaria* spp. But it should be pointed out that these mesocercariae may have been overlooked since they are found subcutaneously in the hind legs of the frog, a site which is probably not examined often.

Disposition of Mesocercariae in Tadpole and Frog. In tadpoles of all 3 species, unencysted mesocercariae were found subcutaneously and in the gelatinous fin of the tail, and subcutaneously over the posterior part of the ventral surface of the body. Mesocercariae were not found in the body cavity. In a frog, *Rana clamitans,* and a toad, *Bufo americanus,* which had been exposed as tadpoles to cercariae and examined shortly after metamorphosis when they still had tail stumps, unencysted mesocercariae were found subcutaneously and inter-muscularly throughout the length of the hind limbs, especially distally.

From this it appears that mesocercariae of *Strigea elegans,* like those of *Alaria canis* and *A. arisaemoides* (see Pearson, 1956), move from the tail and body into the hind limbs when the host undergoes metamorphosis, and that they do not encyst or become encapsulated in the amphibian host.

Tetracotyle (Figs. 11–15)

The diagnosis and description of the tetracotyle are based on a study of living material and of stained whole mounts, both ventral and lateral; measurements (average and range) in microns are given for 15 whole mounts.

Diagnosis: A typical tetracotyle, with large cupuliform forebody feebly marked off from small, conical hindbody; total length 325 (290–380) and width 180 (150–230). Opening of cavity oblique, opening antero-ventrally, as in adult, with ventral margin between level posterior border of pseudosuckers and mid-point of ventral sucker. Dorsal margin of forebody trilobed (especially in contracted state), with median lobe bearing the oral sucker. Oral sucker terminal; length 46 (42–56) and width 45 (42–48). Pharynx small, contiguous with oral sucker; length 23 (21–25) and width 21 (19–23). Esophagus very short; ceca thin-walled, inflated, passing back lateral to ventral sucker and holdfast almost to posterior end. Ventral sucker large and powerful; length 57 (48–69) and width 57 (50–65). Holdfast large, muscular; in form of 2 bipartite lobes when retracted, with a smaller, anterior dorsal lobe and a larger posterior ventral lobe; lobes of holdfast capable of protraction through mouth of forebody cavity; length of retracted holdfast 77 (52–96) and width 75 (69–92). Holdfast gland large, transversely elongate, situated posterior to ventral lobe of holdfast; length 19 (15–23) and width 58 (52–68). A pair of large, glandulo-muscular pseudosuckers, or sucking cups, usually postero-lateral to oral sucker. Genital rudiment small, undifferentiated, lying posterior to holdfast gland in hindbody. Pattern of excretory system not worked out; excretory pore terminal; bladder bicornuate; reserve excretory system well developed, with large chambers packed with excretory corpuscles.

Hosts: garter snake, *Thamnophis sirtalis* (L.), experimental; domestic duck, *Anas boschas* L., experimental and great horned owl, *Bubo virginianus* (Gmelin), experimental. U. S. Nat. Mus. Helm. Coll. No. 38382, from duck.

Remarks. The oral sucker is terminal and opens ventrally. Within it, in living material, can be seen a semi-circle of unicellular gland cells which open at the margin of the oral opening by long ducts (Fig. 13a & b). In fixed material droplets of secretion can be seen at the openings of the ducts. The elongate, cell bodies lie in the dorsal part of the oral sucker, and the fine ducts run circumferentially near the outer limiting membrane of the oral sucker to the margin of the oral opening. These, or similar, glands were not seen in either the cercaria or the mesocercaria; unfortunately, when these gland cells were discovered in the tetracotyle, no living material of cercarial or mesocercarial stages was left to check this point.

Gland cells within the oral sucker do not appear to have been described for any other tetracotyle. Gland cells similarly arranged have been seen by the writer (unpublished) in a tetracotyle from a naturally infected fish taken in Brisbane. Unicellular glands within the oral sucker have been described and figured for the mesocercaria of *Alaria arisaemoides* by Pearson (1956); these gland cells differ in that they open apparently into the oral cavity and not on the margin as they do in the

tetracotyle. Such glands were not found in the diplostomulum stage, that is, the stage comparable with the tetracotyle of *Strigea elegans*, but similar gland cells were found in the oral sucker of the cercaria and these in turn appear to be related to the gland cells described by Looss (1896) and by Langeron (1924) in the oral sucker of *Cercaria vivax*, the cercaria of *Szidatia joyeuxi* (Cyathocotylidae).

It seems unlikely that these oral sucker glands serve the same function in cercariae, mesocercariae, and tetracotyles, since cercariae and mesocercariae are stages adapted for penetration into the tissues of a host whereas tetracotyles are adapted for maturing and feeding in the gut of a host. The oral sucker glands of the tetracotyle may assist in feeding, perhaps by causing external digestion; on the contrary, these glands may be cystogenous and secrete the hyaline, tough material of the parasite cyst (see below). In this connection, it may be noted that the parasite cyst is appreciably thicker over the anterior end of the tetracotyle than elsewhere. If it is demonstrated that tetracotyles can re-establish themselves in paratenic hosts, then it may be found that the oral sucker glands are homologous in function in cercariae, mesocercariae, and tetracotyles, and that their secretion aids in penetration.

The ventral sucker is variable in position, and may be anterior to the holdfast or be superimposed on it ventrally. The ventral sucker can be everted and protracted out of the forebody cavity on a bulbous extension of the forebody wall (Fig. 15). As this bulbous extension stains lightly and seems to be largely empty of parenchyma, it appears that it is formed by the distension of vessels (spaces) of the reserve excretory system dorsal to the ventral sucker. This ability to evert and protract the ventral sucker may aid in attachment to the intestinal mucosa of the final host by drawing a plug of mucosa into the cavity of the forebody.

The paired glandulo-muscular areas on the ventral face of the dorsal wall of the forebody are variable in shape and position, depending on the amount of extension of the adjacent dorso-lateral body wall (Fig. 14a–d). Structurally, these organs are roughly circular areas of the ventral body wall modified by a greater muscularity and by the presence of the openings of many unicellular glands. The bodies of these gland cells lie beneath the muscle layers, parallel to the body surface, are elongated antero-posteriorly, and open to the surface by short, vertical ducts. Retraction of the organ is effected by a band of muscle fibers running back in the parenchyma. The mechanism of protraction was not apparent, but may be effected by a squeezing of fluid into the channels of the reserve excretory system of the organ.

As the pseudosuckers and lappets of strigeoids are homologous organs, it is unfortunate that there is not a single and collective name, especially as this organ can assume both forms in some species: e.g. tetracotyle of *Strigea elegans* described here; *Proalaria huronensis* (= *Diplostomum huronense*) described by La Rue (1927); diplostomulum of *Alaria arisaemoides* described by Pearson (1956); and adults of *Alaria mustelae* described by Bosma (1934) and observed by the writer, and of *Alaria taxideae* examined by the writer.

Cyst. (Fig. 12). Tetracotyles recovered from experimentally infected birds were both encysted and encapsulated. The cysts were surrounded by a thin layer of host fibrous tissue which fastened them to the subcutaneous loose connective tissue and muscle fascia. Fifteen cysts from a duck infected 22 days before measured 520 (460–630) microns by 380 (300–470) microns, and the inner cyst measured 460 (430–490) microns by 240 (230–270) microns. The cyst wall is made up of 2 main parts, an outer, thick layer and an inner, thin layer (Fig. 12). The outer layer

is translucent, gelatinous or rubbery, varies from 85 to 130 microns in thickness, and is composed of concentric layers of homogeneous material alternating with layers of fibers in bundles forming a feltwork. This outer layer is subdivided into 2 layers of about equal thickness, an outer half with flexible, non-granular matrix containing scattered clumps of corpuscles and granules and an inner half with somewhat friable, granular matrix. Neither leucocytes nor their remains were seen in the cyst wall. The inner layer of the cyst wall is tough, hyaline, yellowish, transparent, about 5–6 microns thick, and thickened (10 microns) over the anterior end of the tetracotyle. When cysts were teased apart, the outer half of the outer cyst wall peeled off in concentric layers, and the inner half tended to come away in irregular pieces, which however suggested concentric layering. The inner cyst wall split open, but recovered and retained its shape after the tetracotyle was removed. In an intact cyst, the contracted tetracotyle lies uncrowded in a thick, clear fluid containing released excretory granules. No passages through the cyst wall were found such as have been described for the cyst of *Tetracotyle typica* (= *Cotylurus cornutus*) by Szidat (1924).

According to Hughes (1928a), the outer cyst wall is of host origin and the inner cyst wall of parasite origin in the cyst of *Tetracotyle pipientis* Faust. But Olivier (1940), who studied the development of this same form (= tetracotyle of *Apharyngostrigea pipientis*), found that the whole of the cyst wall is of parasite origin. Earlier, Szidat (1924) had found that the outer cyst wall was secreted by the developing tetracotyle of *Cotylurus cornutus*.

The cyst described above, with its thick, outer layer and thin, hyaline inner layer is like that described for most tetracotyles, for example, *Tetracotyle communis* (= *Cotylurus communis*), *T. diminuta*, and *T. intermedia* described by Hughes (1928b); *T. bonasae* described by Chandler (1954); and the tetracotyle of *Strigea vaginata* described by Lutz (1933a). In the descriptions of some cysts, no mention is made of an inner hyaline layer; but it is difficult to decide whether this layer was absent or overlooked. Hughes (1929), in describing the cyst of *Tetracotyle serpentis*, says that the hyaline layer is surrounded by a thin layer of host connective tissue; that is, that there is no gelatinous layer.

Related Species. On the basis of the life cycle described here and that described by Lutz (1933a), one would expect to find the most closely related tetracotyles parasitic in reptiles, birds, and mammals, considering as doubtful Lutz's claim that fish and frogs also act as hosts. A list of such tetracotyles is given below. It seems probable that all of these forms are larvae of species of *Strigea*, or perhaps *Apatemon* (*Pseudostrigea*), at any rate of strigeids parasitic in raptorial birds.

1. *T. bonasae* Chandler, 1954 in ruffed grouse, *Bonasa umbellus*.
2. Tetracotyle of *Strigea vaginata* by Lutz (1933 a) in birds (terns); canid, *Thous cancrivorus* (= *Canis azarae*); and mustelid, *Grisonia vittata* (= *Galictis vittata*).
3. *T. tiliquae* Nicoll, 1914 in blue-tongued lizard, *Tiliqua scincoides*.
4. *T. serpentis* Hughes, 1929 in garter snake, *Thamnophis s. sirtalis* (L.) and water snake, *Natrix s. sipedon* (L.).
5. *T. foetorii* von Linstow, 1876 in ferret, *Foetorius putorius* (= *Mustela putorius* L.).
6. Tetracotyle of *Strigea strigis* in European snakes. (see Hughes, 1929 and Dubois, 1938).
7. *T. soricis* von Linstow, 1877 in the shrew, *Sorex vulgaris*.
8. *T. bicolandiae* Tubangui, 1933 in the tern, *Sterna sinensis*.

Of the above tetracotyles, only *T. bonasae* and *T. serpentis* are described in sufficient detail to warrant comparison. *T. bonasae* is morphologically similar to the tetracotyle of *Strigea elegans* (indeed tetracotyles generally appear to be uniform

morphologically), but differs in being almost twice as large, but with proportionally smaller oral and ventral suckers and wider holdfast. *T. serpentis* closely resembles the tetracotyle of *Strigea elegans* in measurements of body parts, but differs in having a thinner cyst wall which is only 1/2 to 1/3 as thick.

SUMMARY

1. *Strigea elegans* is described from a new host, the snowy owl (*Nyctea nyctea* L.).

2. The snail, *Gyraulus parvus,* served as experimental first intermediate host. Cercariae first emerged 26 days after exposure.

3. Tadpoles of the American toad, *Bufo americanus,* wood frog, *Rana sylvatica,* and green frog, *Rana clamitans,* served as experimental second intermediate hosts. In the tadpoles, cercariae developed into mesocercariae in about 2 weeks.

4. When mesocercariae were fed to a garter snake, *Thamnophis sirtalis,* and to domestic ducklings, *Anas boschas,* they developed into encysted, encapsulated tetracotyles in less than 21 days.

5. When mesocercariae were fed to 2 fledgling great horned owls, *Bubo virginianus,* they apparently developed into tetracotyles since no eggs were found in the feces and no adult worms were found in the intestines of the owls, whereas tetracotyles were found in the tissues.

6. It appears, then, that *Strigea elegans* has an obligatorily 4-host life cycle.

7. The morphology of adult, cercaria, mesocercaria, and tetracotyle is described and observations are given on the escape of cercariae from the snail host and on the migration of mesocercariae during metamorphosis of the tadpole host.

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List of Abbreviations

Bursa	genital bursa
Diag. Musc.	diagonal muscle fibres
Dors. Lobe	dorsal lobe of holdfast
Egg	egg in uterus
Ej. Duct	ejaculatory duct
Excr. Atr.	excretory atrium
Excr. Bl.	excretory bladder
Excr. Space	space of reserve excretory system
Ext.	extension of ventral body wall
Gen. Cone	genital cone
Gen. Rud.	genital rudiment
Gran.	granules
H. Gl.	holdfast gland
Inner Cyst	inner, thin, hyaline cyst
Inner Half	inner half of outer cyst wall
Lat. Marg.	lateral margin of forebody cup
Lat. Musc.	muscle fibres running from ventral sucker to lateral body wall
L.H.	lobes of holdfast
M. Gl.	Mehlis' gland
Outer Half	outer half of outer cyst wall
Ov.	Ovary
Ps.	pseudosucker
Ringn.	Ringnapf
Sec. H. Gl.	secretion of holdfast gland
Sem. Ves.	seminal vesicle
T_1	anterior testis
T_2	posterior testis
Ut.	uterus
Vas. Def.	vas deferens
Vent. Lobe	ventral lobe of holdfast
Vent. Marg.	ventral margin of forebody cup
Vit. Foll.	vitelline follicle
Vit. Res.	vitelline reservoir
V.S.	ventral sucker

Description of Plates

(Figures drawn with aid of *camera lucida* unless otherwise stated)

Plate I *Adult*

Figure 1. Lateral view of adult *Strigea elegans* from *Nyctea nyctea*. U. S. Nat. Mus. Helm. Coll. No. 38381.

Figure 2. Bursal region of adult, based on reconstruction of serial sagittal sections.

Figure 3. Ventral view of dorsal part of forebody of adult, showing pseudosuckers (ventral part of forebody and lobes of holdfast removed).

Plate II *Cercaria*

Figure 4. Dorsal view of body of cercaria (outline by *camera lucida*).

Figure 5. Ventral view of cercaria, showing arrangement of spines, caudal supporting cells, and caudal excretory tubules.

Figure 6. Movements of oral sucker; (a) extended, (b) oral opening invaginated, (c) oral sucker partly retracted, and (d) oral sucker fully retracted and oral opening further invaginated.

Figure 7. Freehand sketch of cercaria; (a) position at cessation of swimming (b) with furcae extended, (c) resting position.

Plate III *Mesocercaria*

Figure 8. Ventral view of mesocercaria, showing spination, gut, and diagonal muscle fibres.

Figure 9. Dorsal view of mesocercaria, showing excretory system and penetration glands.

Figure 10. Variations (a and b) in rudiment of reserve excretory system.

PLATE IV *Tetracotyle*

FIGURE 11. Ventral view of tetracotyle. U. S. Nat. Mus. Helm. Coll. No. 38382.
FIGURE 12. Tetracotyle in cyst (from a fresh specimen with cyst wall somewhat flattened under a coverslip).
FIGURE 13. Gland cells in oral sucker: (a) ventral view, (b) optical sagittal section, (free hand sketches).
FIGURE 14. Variations (a–d) in form of pseudosuckers and adjacent ventral body wall.
FIGURE 15. Lateral view of a tetracotyle with everted, protracted ventral sucker.

RESEARCH NOTE

IS *CHILOMASTIX* A PATHOGEN?

Perusal of nine widely used textbooks of medical parasitology reveals the unanimous opinion that while *Chilomastix* is usually found in diarrheic stools, it is a harmless commensal and not responsible for symptoms. As Napier (1946, The Principles and Practice of Tropical Medicine) expresses it: "It would be absurd for the writer to describe a symptomatology which he does not believe exists." In further confirmation of this attitude, no treatment is prescribed. This position has recently been questioned by Cerva and Vetrovska (1958, Ceskoslov. Epidemiol., Mikrobiol., Immunol. Prague **7**: 126–135. Reviewed in Trop. Dis. Bull. Nov., 1958). These authors concluded that *Chilomastix* infection was responsible for a periodic watery diarrhea occurring in a group of very young institutionalized children in Prague, and observed marked improvement and weight gain following treatment with carbarsone. The temperature of the children was not raised, and the general condition unaffected.

In light of the foregoing, the following personal experience is of interest. During the middle of November, 1958, the writer spent a week in the Peten region of northern Guatemala, living at Flores and at various points in the immediate vicinity of Lake Peten. Sanitation of the region is primitive or non-existent, and the usual precautions prove impractical when one is dependent upon local accommodations for meals. Several days after returning to this country (Nov. 21), a diarrhea developed, which progressively became more and more watery, until finally there was some difficulty in retaining the bowel contents. As many as 8 or 10 liquid stools were passed in a day. There was no pain or tenderness, and the only other symptom was a loss of appetite, with perhaps a very slight feeling of nausea. On November 27 a stool examination was made for the first time, and motile *Chilomastix* were discovered, about 12 to 15 per high dry field. On November 28 and 29 there occurred a rapid increase in numbers, until on the latter date the field of the microscope was swarming with motile trophozoites, and the stool appeared to be about 50% flagellates. Fewer organisms were observed in a liquid stool on November 30, but at the time this was attributed to greater dilution of the specimen for examination. No bowel movement occurred on December 1. On December 2 the stool was soft, but formed, and repeated examination revealed no trace of *Chilomastix*. Since that date the stools have been normal, and repeated examination has shown no further evidence of flagellates, either motile or cysts.

Since no drug was taken at any time, the infection must have undergone spontaneous cure. No cysts were found at any time. The infection was verified by various members of a city laboratory. There is, of course, no way of proving that *Chilomastix* was not superimposed upon a diarrhea of different origin, especially since no stool cultures were made. However, the rise and fall of the flagellate population coincided with the course of symptoms exactly, and suggested a causal relationship. The occurrence of spontaneous cure indicates that the infection must have an effect upon the host, otherwise it becomes difficult to explain a host response sufficient to eradicate the infection. If the organism were a simple commensal, there is no reason why it should not persist indefinitely, once having reached an equilibrium. It is difficult to explain the disappearance of the organism on the basis of a change of diet after having returned home, since the entire course of the infection and symptoms (except for the initial inoculation) occurred after returning to customary surroundings. Perhaps further thought should be directed toward the possibility that *Chilomastix* may be a potential, if mild, pathogen.—JUSTUS F. MUELLER, *State University of New York, Upstate Medical Center, Syracuse 10, N. Y.*

PLATE I

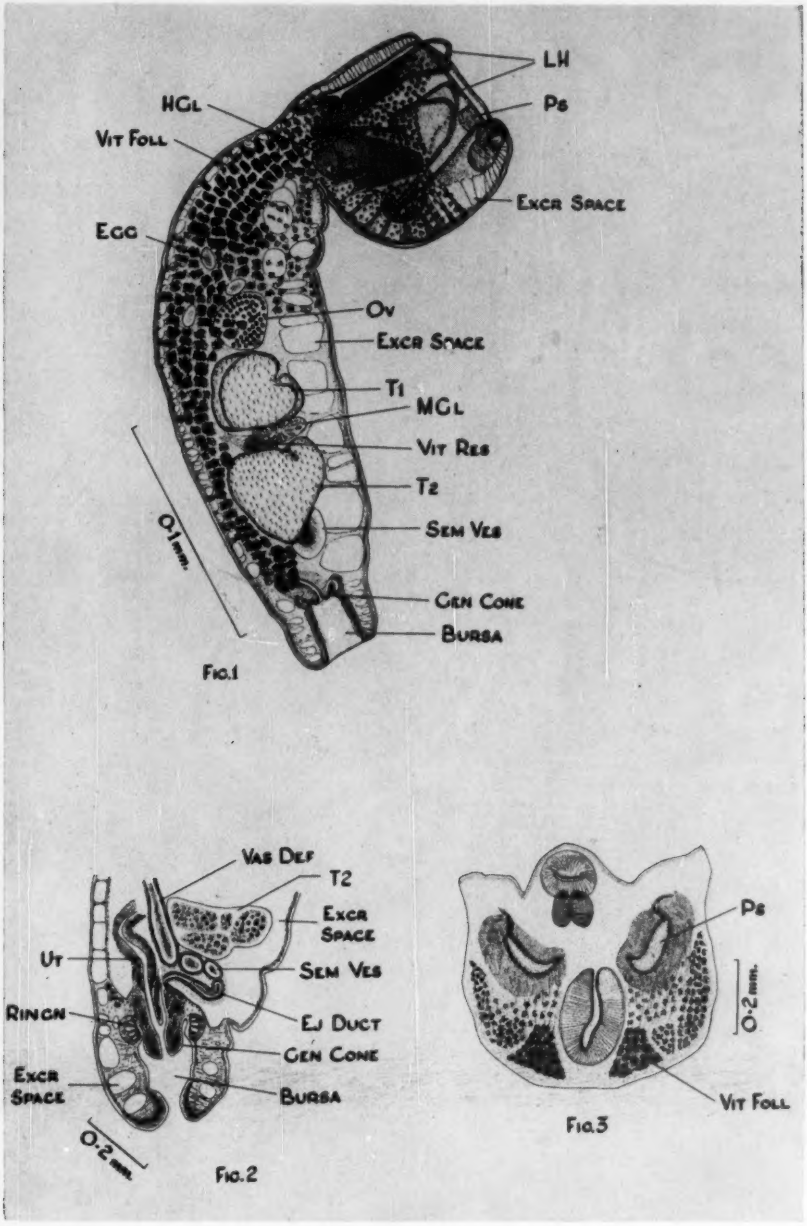
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FIG.3

PLATE II

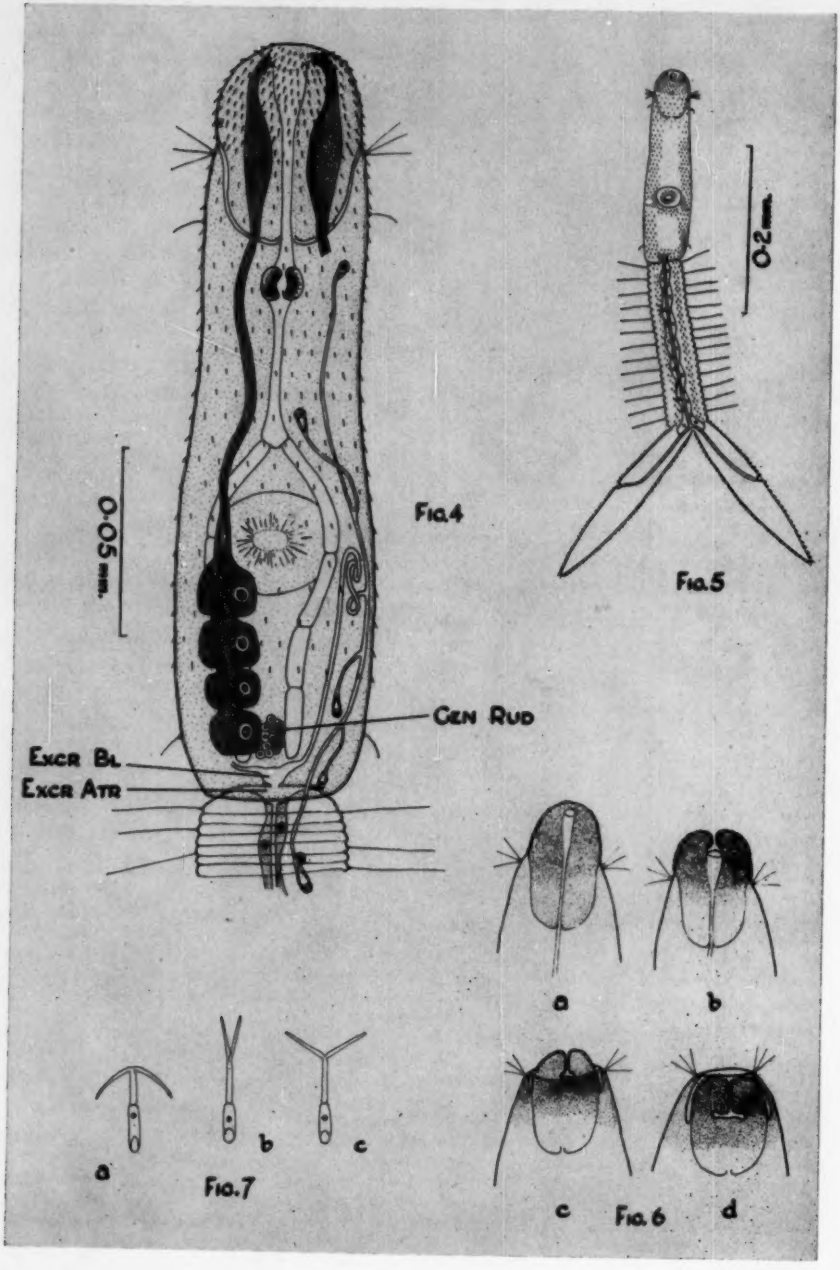
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FIG. 6

PLATE III

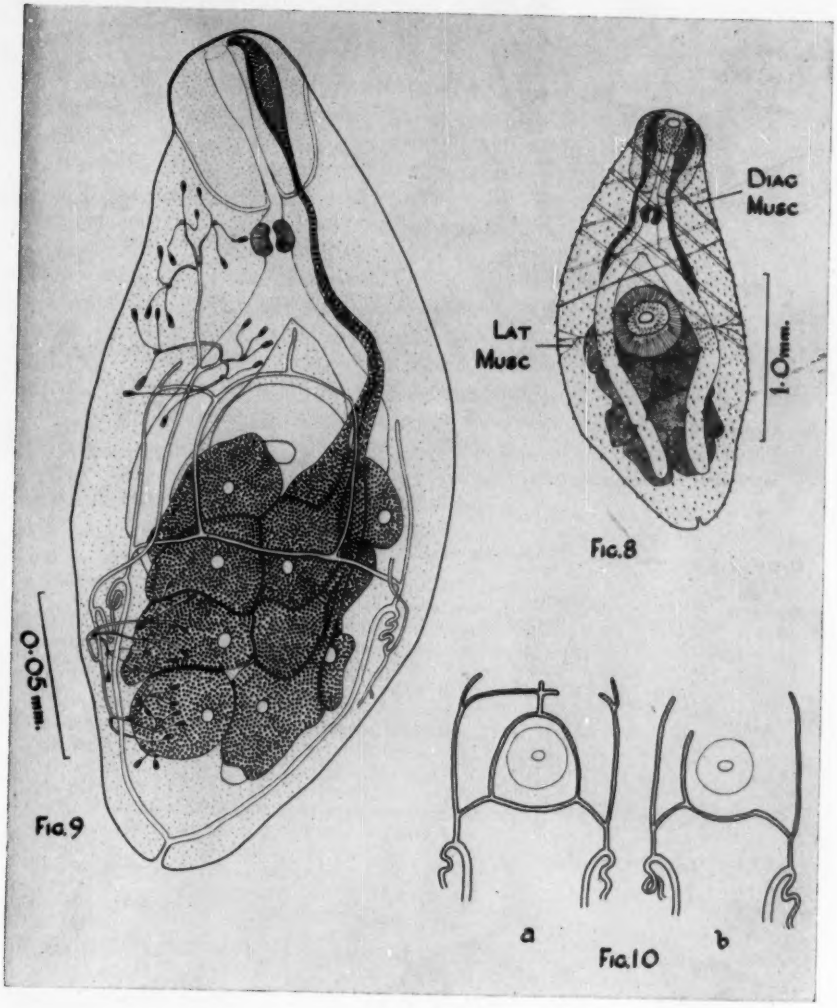

PLATE IV

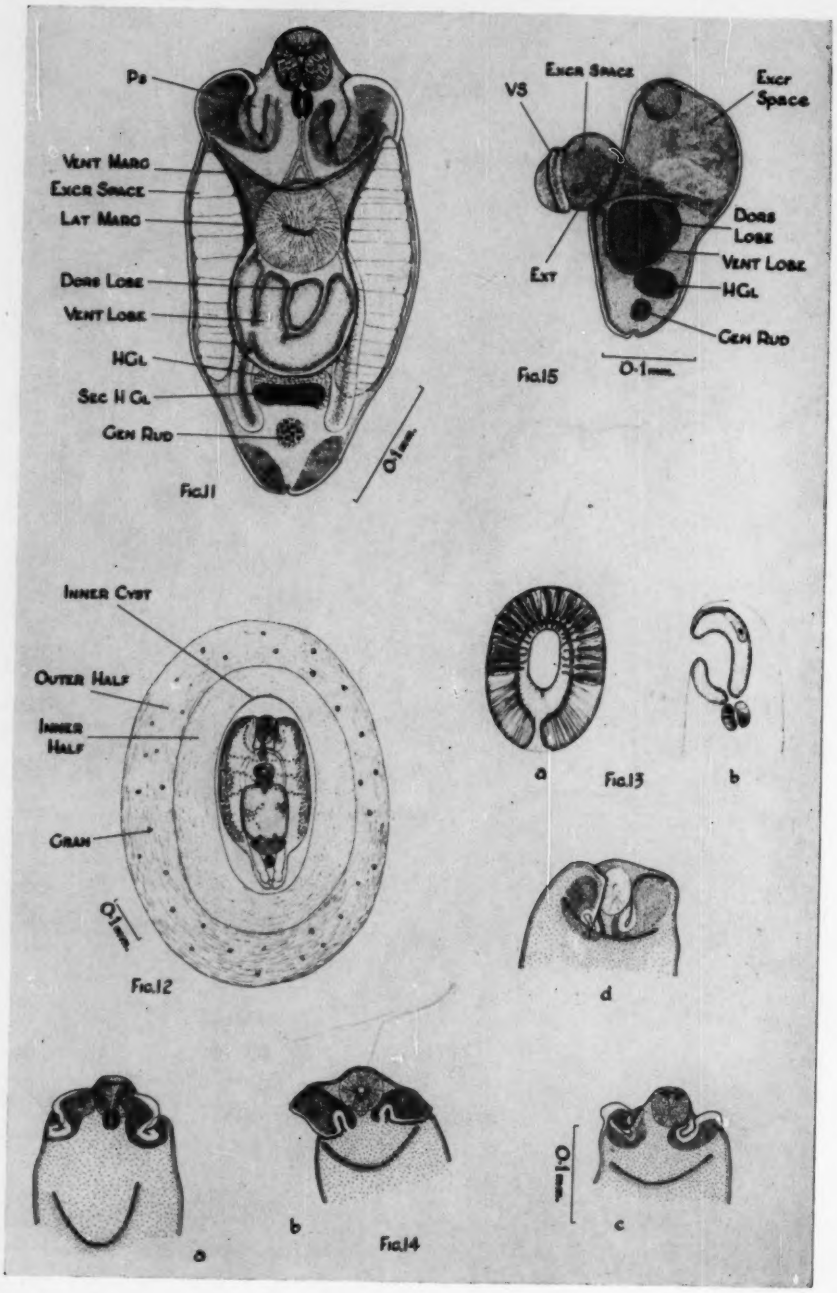
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Fig.11
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Gen Rud
0·1 mm.
Fig.15
Inner Cyst
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Fig.12
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Fig.14
0·1 mm.
c

SENSITIVITY OF DEVELOPING *HYMENOLEPIS DIMINUTA* LARVAE TO HIGH TEMPERATURE STRESS*

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The purpose of the studies reported here was to determine if differences in sensitivity to high temperature exist at different stages in the larval development of the cestode *Hymenolepis diminuta.* Voge and Heyneman (1958) have described structural abnormalities which occur in these larvae when grown in confused flour beetles at supraoptimal temperatures of 38.5 and 40° C. Voge and Turner (1956) showed that cysticercoid development of *H. diminuta* may proceed normally at temperatures as high as 37° C. In all the above studies, larvae were kept at 37° C or higher for their total period of development and growth. It was therefore of interest to establish as precisely as possible: 1) The period of exposure to supraoptimal temperature which would cause abnormal development, 2) The stage or stages during larval development most sensitive to such exposure, 3) The relation between length of exposure during the developmental period and incidence of abnormalities, and 4) The effect, if any, of relatively brief exposure on infectivity of cysticercoids to the final host. Furthermore, it was thought desirable to compare the results obtained from such studies with information available on high temperature sensitivity during development of some free-living invertebrates.

MATERIALS AND METHODS

Animals used in this study were the Rice Institute strain of *Hymenolepis diminuta,* laboratory-raised *Tribolium confusum* maintained in enriched flour, and Wistar albino rats.

Infection of beetles was carried out as described by Voge and Heyneman (1957), except that beetles were allowed to feed on tapeworm eggs for 6 hours only. All infected controls were kept at 30° C. Experimental groups were placed in appropriate incubators at different stages of cysticercoid development. In different groups, exposure to high temperature varied from 6 hours to 4 days. At all other times, experimentals were kept at 30° C. Beetles were dissected 8 or 16 days after infection; all larvae were counted and observations on their general appearance recorded. Larvae were then fixed in Bouin's fluid and stained with a very dilute solution of Ehrlich's hematoxylin, or they were fed to rats. Only normal-appearing, fully withdrawn larvae were used in infection experiments with rats.

All experiments described here were repeated many times. Care was taken to record separately results obtained from those beetles which were heavily infected so as to rule out the effects of crowding. Unless otherwise stated, all data presented below are based on moderate infections (10 or fewer cysticercoids per beetle).

EXPERIMENTAL RESULTS

The normal sequence of cysticercoid development at 30° C is shown diagrammatically in Figure 1. The egg of *H. diminuta,* when ingested by the flour beetle, develops into an infective cysticercoid in 8 days. As can be seen, the major amount of growth and development of the larva occurs between the 2nd and 6th day. If growing larvae are exposed for 6 hours at 40° C at any stage of their development,

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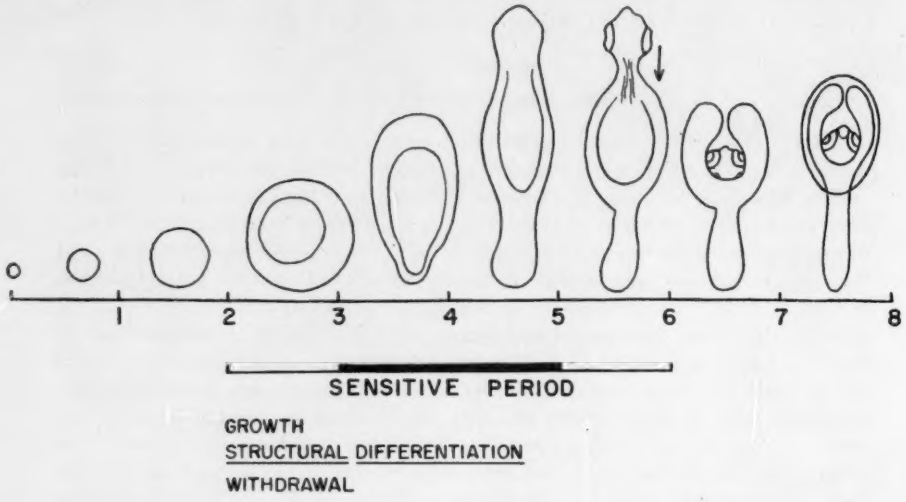
PATTERN OF DEVELOPMENT OF *HYMENOLEPIS DIMINUTA* LARVAE AT 30°C



apparently no deleterious changes are produced. Some of the results obtained from 24-hour exposure of developing larvae to 38.5° or 40° C are shown in Figure 2. The scale on the left column represents the developmental span. The time and stage of exposure to high temperature is represented by the solid black area. The two indicators of sensitivity are failure of scolex-withdrawal and loss of infectivity of withdrawn and structurally normal larvae. Failure of scolex-withdrawal had been observed previously in some larvae grown for the total period of development at 37° C (Voge and Turner, 1956) and in all larvae grown at 38.5° C throughout the developmental period (Voge and Heyneman, 1958). Figure 2 shows clearly that the most sensitive period is between the 3rd and 5th day. Incidence of inhibition of scolex-withdrawal is highest when exposure occurs from days 3–4; infectivity of withdrawn larvae from the same group is reduced. If exposed for 24 hours on days 4–5, larval structure is affected only to a slight degree but infectivity to the final host is inhibited. The total sensitive period extends from days 2–6.

If the period of exposure to 38.5° or 40° C is increased to 2 or to 4 days (Fig. 3), one observes results essentially similar to those obtained with 24-hour exposure except that the incidence of morphological abnormalities, that is failure of scolex-withdrawal, is increased. When exposure has occurred for 4 days, infectivity of withdrawn larvae has been completely suppressed. Similarly, exposure during days 3–5 also suppresses infectivity, while exposure during days 4–6 decreases the rate of infectivity. Failure of scolex-withdrawal is highest when exposure has occurred at days 3–5. While increased length of exposure generally produces a greater incidence of structural abnormality, it can be seen that exposure has to occur during the sensi-

EFFECT OF 24 HOUR EXPOSURE OF DEVELOPING *H. DIMINUTA* LARVAE TO SUPRA-OPTIMAL TEMPERATURES

PERIOD OF EXPOSURE IN DAYS	38.5° C		40° C		INFECTIVITY	
	NOT WITHDRAWN	WITHDRAWN	NOT WITHDRAWN	WITHDRAWN	38.5° C	40° C
1 2 3 4 5 6 7 8	1 (0.96%)	103 (99%)	0 (0%)	49 (100%)	+	+
1 2 3 4 5 6 7 8	0 (0%)	35 (100%)	0 (0%)	64 (100%)	+	+
1 2 3 4 5 6 7 8	5 (8%)	56 (92%)	9 (24%)	29 (76%)	+	+
1 2 3 4 5 6 7 8	15 (48%)	16 (52%)	61 (75%)	20 (25%)	±	±
1 2 3 4 5 6 7 8	2 (3%)	66 (97%)	8 (8%)	87 (92%)	−	−
1 2 3 4 5 6 7 8	9 (17%)	43 (83%)	1 (3%)	30 (97%)	+	+
1 2 3 4 5 6 7 8	0 (0%)	46 (100%)	0 (0%)	32 (100%)	+	+

FIGURE 2. Scale to the left represents developmental period in days. Black areas indicate time of exposure to high temperature. Results obtained by exposure on days 3–4 and 4–5 show that this is period of greatest sensitivity.

tive period, since incidence of abnormals is higher at 2-day exposure (3–5) than at 4-day exposure (1–4). However, as exposure during the first 4 days completely suppresses infectivity, it would seem that the factors or processes determining infectivity differ from those determining scolex-withdrawal. This is also borne out by results obtained from 24-hour exposure at days 3–4 and 4–5.

In order to determine the effect of high temperature on fully developed, normal cysticercoids, beetles containing larvae developed at 30° C several weeks previously, were exposed to 40° C for 4 days. The cysticercoids were then dissected out in the usual manner, examined under the microscope and fed to rats. Not only were all these larvae normal in structure, but as seen from autopsy of rats 20 days later, they were also as infective as were untreated controls. This clearly demonstrates that the influence of high temperature which is deleterious during certain critical stages of development may be harmless for the fully developed organism.

Because previous studies (Voge and Heyneman, 1958) had shown that growth under high temperature could delay development of *H. diminuta,* it was thought that larvae which had failed to withdraw the scolex after 8 days' development would perhaps do so if allowed to remain in the beetle hosts at 30° C for an additional 8 days. Therefore, all experiments shown in Figures 2 and 3 were accompanied by experimental sets which were dissected at the end of 16 instead of 8 days. However, the results obtained from these larvae closely paralleled the data shown in Figures 2 and

EFFECT OF EXPOSURE OF DEVELOPING H. DIMINUTA LARVAE TO SUPRA-OPTIMAL TEMPERATURES

PERIOD OF EXPOSURE IN DAYS	38.5° C		40° C		INFECTIVITY	
	NOT WITHDRAWN	WITHDRAWN	NOT WITHDRAWN	WITHDRAWN	38.5° C	40° C
1 2 3 4 5 6 7 8	53 (49%)	55 (51%)	62 (49%)	65 (51%)	-	-
1 2 3 4 5 6 7 8	28 (43%)	37 (57%)	107 (84%)	20 (16%)	-	-
1 2 3 4 5 6 7 8	1 (2%)	56 (98%)	3 (2%)	131 (98%)	+	+
1 2 3 4 5 6 7 8	33 (37.5%)	55 (62.5%)	58 (40%)	88 (60%)	+	-
1 2 3 4 5 6 7 8	93 (74%)	33 (26%)	140 (72%)	54 (28%)	-	-
1 2 3 4 5 6 7 8	55 (35%)	104 (65%)	28 (50%)	28 (50%)	±	±
1 2 3 4 5 6 7 8	2 (6%)	30 (94%)	13 (11%)	100 (89%)	+	+
1 2 3 4 5 6 7 8	2 (2%)	95 (98%)	1 (2%)	44 (98%)	+	+

FIGURE 3. Scale to the left represents developmental period in days. Black areas indicate time of exposure to high temperature. Note that the highest rate of abnormalities is obtained from exposure at days 3–5 which is the sensitive period for the characters analyzed.

3: Incidence of non-withdrawn larvae was similar, as was infectivity of withdrawn larvae. This suggests that each developmental process can occur only at a given time during development. Once this time has passed, "repair" is not possible, at least under the conditions used here.

The obvious question of whether or not high temperature was lethal to larvae exposed during the most sensitive period arose. Comparison of stained larvae obtained after 8 and 16 days shows that growth of certain structures proceeds after exposure for 4 days to 40° C, and that this growth occurs in both withdrawn and non-withdrawn larvae. Figure 4a shows a non-withdrawn larva which had been exposed to 40° C for 2 days (days 2–4) and removed from the host after 8 days. Figure 4b shows a larva from the same experiment dissected from the host after 16 days. In this larva, normal growth and development of the external membrane has occurred as it would in any normal and withdrawn cysticercoid. Furthermore, the musculature of the suckers has become better defined. Similar development has been observed in withdrawn larvae from the same experiment, and in larvae of all other experiments as well. It appears, therefore, that high temperature, while producing structural as well as physiological abnormalities, does not necessarily kill the larvae.

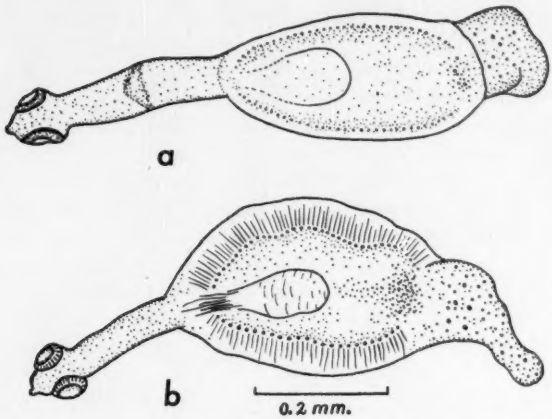


FIGURE 4, a. Larva of *Hymenolepis diminuta*, removed from host after 8 days; scolex-withdrawal has been inhibited by exposure to 40° C at days 2–4 of development. Note absence of definite sucker musculature and lack of fibers in external membrane. b. Larva from same experiment, dissected from host after 16 days. Note additional development of sucker musculature and external membrane.

Microscopic examination of withdrawn larvae which had been exposed to high temperature during development showed surprisingly few gross abnormalities. Of these, unequal growth along the longitudinal axis was seen most frequently. In a few the scolex appeared to be slightly twisted. The majority, however, was indistinguishable from normal controls. In this connection, it should be emphasized that normal morphology does not in any way predicate infectivity to the final host, although abnormal morphology usually precludes infectivity.

DISCUSSION

While numerous studies have been conducted on the influence of environmental temperature upon vertebrates and invertebrates, relatively few experiments have dealt with the effect of high temperature on development of invertebrates. Most important among the latter are studies performed on insects, particularly the fruit fly, *Drosophila*. One might suppose that the great disparity between *Drosophila* and *Hymenolepis diminuta* would not allow for the possibility of valid comparison of effects produced by exposure to high temperature. However, some of the results obtained indicate that, in general, the reactivity of these two organisms is basically similar after exposure to high temperature during different stages of development. Henke, et al (1941) observed in their studies with *Drosophila* that total time of development is increased by exposure to high temperature; this was found to be true for *H. diminuta* and *H. nana* (Voge and Heyneman, 1958). Henke et al also established the presence of sensitive periods for certain modifications which could not be produced at periods other than the sensitive period. This is in general agreement with results reported for *H. diminuta* in which the most sensitive period lies between days 3–5 of development.

Precht et al (1955; pp. 128–129) summarize the general effects of high temperature on the development of some insects and state that adverse effects are greatest

when exposure occurs during periods of extensive development and growth. They also stress that the magnitude of effects elicited differs at different times during the life of the organism. The results obtained with *Hymenolepis diminuta* confirm these observations in that exposure must occur during the sensitive period which coincides with maximal development and growth (see Figs. 1 and 2). If stress is applied at other periods, the length of exposure must be increased. That different developmental processes have different sensitive periods is seen from results obtained with inhibition of scolex withdrawal and infectivity in *H. diminuta*. These processes appear to be independent of one another and have slightly different although somewhat overlapping sensitive periods. In general, it would appear that, regardless of differences in complexity or affinity, the effect of high temperature and the response of the various organisms during development is similar, at least in principle.

With regard to the observations on *Hymenolepis diminuta*, many new questions and problems have arisen. One of these concerns inhibition of infectivity in larvae which appear to be alive and structurally normal. Is it possible that alteration of the environment of the definitive host would permit establishment of these larvae which are perhaps only weakened and may require a longer time for excystation and attachment than is available under normal conditions? Another problem concerns the mechanism of scolex-withdrawal of developing larvae. Is inhibition of withdrawal simply due to dehydration and if so, could increased humidity prevent the occurrence of this abnormality? The possibility of preventing the effects of exposure to high temperature by modifying other factors of the environment in which development proceeds, will be investigated.

SUMMARY

Larvae of *Hymenolepis diminuta* growing at 30° C in *Tribolium confusum* were exposed to temperatures of 38.5° or 40° C at different stages of their development. Length of exposure varied from 6 hours to 4 days in different experiments.

Major indicators of sensitivity to high temperature are failure of scolex-withdrawal and inhibition of infectivity of normally withdrawn larvae for the mammalian host. Experiments with temperature stress lasting 24 hours or longer showed the following:

The presence of a sensitive period extending from days 2–6 of larval development with maximum sensitivity during days 3–5.

This sensitive period coincides with maximum larval growth and development. Exposure during this period results in failure of scolex withdrawal of a certain number of larvae and in inhibition of infectivity of normal, withdrawn larvae. The sensitive periods for scolex-withdrawal and infectivity apparently overlap but do not coincide.

Exposure to high temperature, which produces abnormalities during the sensitive period, has little or no effect when applied at other times during development. Fully developed cysticercoids are not affected even by exposures which, because of long duration, always prevent infectivity in the developing larva.

Results obtained with *Hymenolepis diminuta* are discussed and compared with information available on high temperature sensitivity for some other free-living invertebrates.

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RESEARCH NOTE

TWO CAMPULID TREMATODES FROM A NEW HOST, THE HARBOR PORPOISE

During the summer of 1958 a young male harbor porpoise, *Phocaena vomerina* (Gill) 1865, was examined by the authors at the Friday Harbor Marine Laboratories at Friday Harbor, Washington.

The fourth chamber of the stomach contained a large number of campulid trematodes which generally agree with the description of *Hadwenius nipponicus* Yamaguti, 1950. The original description was based on 2 specimens from the small intestine of Dall's porpoise, *Phocoenoides dalli* (True) 1885 in Japan.

Over 100 specimens were recovered from the harbor porpoise and measurements of 10 of these, in millimeters, are as follows (average in parentheses): Body slender, spined at anterior end; length, 13 to 15.9 (14); width at level of acetabulum 0.805 to 0.963 (0.886). Oral sucker terminal, 0.776 to 0.891 (0.840) by 0.603 to 0.718 (0.648); acetabulum 0.575 to 0.718 (0.633) in diameter. Prepharynx 0.287 to 0.575 (0.445); pharynx sub-globular 0.316 to 0.431 (0.351) by 0.287 to 0.348 (0.327). Esophagus from practically lacking to 0.203 (in one specimen). Anterior and posterior ceca simple, the latter opening into the excretory bladder. Genital pore immediately in front of acetabulum. Cirrus sac 0.971 to 1.421 (1.257) in length; cirrus opening into genital atrium on the right side, protrusible, with long spines inserted upon basal discs. Anterior testis smaller or equal to posterior testis, the former 0.560 to 0.826 (0.673) by 0.232 to 0.348 (0.290); the latter 0.647 to 0.826 (0.733) by 0.287 to 0.431 (0.344). Intertesticular space present. Ovary almost round, pretesticular 0.174 to 0.275 (0.222) by 0.175 to 0.290 (0.220). Vitellaria in tubular clusters of follicles usually commencing at the level of the middle of the anterior testis, sometimes at the posterior edge of anterior testis, ending near the posterior tip of the body, anteriormost group of follicles often separated from others. Eggs triangular in cross-section, oval, with truncated anterior pole and knobbed posterior pole; 70 to 82 by 35 to 45 μ.

The present material differs from Yamaguti's 2 specimens as follows: 1) Smaller size of body; in fact, the largest specimen (15.9) is smaller than the smaller of Yamaguti's specimens (17.5). The suckers and internal organs are also regularly smaller. The smaller size may be attributed to the heavy infection. 2) Intertesticular space present while in Yamaguti's specimens the testes were contiguous. 3) More anterior extent of vitellaria. The most anterior extent of the vitellaria in Yamaguti's specimens was the posterior edge of the anterior testis. 4) Egg sizes overlap only slightly. Egg sizes in Yamaguti's specimens were 80 to 90 by 45 to 50 microns.

The liver of the same porpoise contained specimens of *Campula oblonga* Cobbold, 1858. These parasites were numerous both immediately beneath the serous membrane and in bile ducts. This species has been previously reported in *Phocaena phocaena* (L.) of Europe and North America and in *Phocoenoides dalli* of Japan.—HILDA LEI CHING AND EDWARD S. ROBINSON, *Zoology Department, University of Nebraska.*

THE SURVIVAL OF MIGRATORY AND POST-MIGRATORY STAGES OF *LITOMOSOIDES CARINII* IN WHITE RATS*

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The filarial worm, *Litomosoides carinii* (Travassos, 1919), Chandler, 1931, occurs naturally in the pleural cavities of wild cotton rats (*Sigmodon hispidus*). Vaz (1934) and Bell and Brown (1945) have reviewed the synonymy of *L. carinii* and list the rodents in which the worm has been naturally found.

Laboratory rodents have been investigated as possible experimental hosts by several workers. Hawking and Burroughs (1946) have reported infection and the production of microfilariae in piebald rats, hamsters, and mice. Since the first report of this infection in white rats by Chandler (1931), several workers have reported on the white rat as a host for *L. carinii* (Scott and Cross, 1946; Scott et al, 1946; Scott, 1947; Williams and Brown, 1946; Williams, 1948; Bertram et al, 1946a, 1946b; Bertram, 1947). These workers appear to regard the white rat as as unsuitable host for this infection.

In the experiments cited above, the infective larvae were introduced into the host following contact with infected mites. Consequently, the number of larvae entering the host was either estimated or unknown. The development by Scott and Macdonald (1953) of a quantitative method of introducing infective larvae afforded an opportunity to test more carefully the comparative resistance of white rats and cotton rats.

MATERIALS AND METHODS

Laboratory-reared cotton rats (*Sigmodon hispidus texianus*) and commercially reared white rats (Carworth-Wistar) were used when approximately 1 year of age (i.e., old rats). Infective larvae were obtained from mites (*Ornithonyssus bacoti*) which had fed on infected-eastern cotton rats (*S. hispidus hispidus*). Infected mites were cultured 20 days before use (Scott, 1952). The method of Macdonald and Scott (1953) was employed for subcutaneous infections.

When worms were transferred from donor to recipient rat, a method reported in a preliminary paper by Olson et al (1954) was employed. The developing worms were recovered in saline from the pleural cavities of subcutaneously infected donor cotton rats. These worms were examined to obtain the desired developmental stage, damaged worms were discarded, and the worms transferred in saline by washing them off a cover slip into the peritoneal cavity of the recipient rat. The cover slip was examined to verify complete delivery of the worms, and the incision sutured in 2 layers with cotton. At the close of an experiment, the pleural and peritoneal cavities of animals were thoroughly washed with saline to recover worms. Encapsulation of worms by host cells was recorded. Each worm was fixed, mounted, measured (Westbrook and Scott, 1955) and examined for morphological development (Cross and Scott, 1947, Scott and Macdonald, 1951).

In experiments on production of microfilariae, a plastic tube of uniform bore was calibrated for volume per unit of length. Measured amounts of blood were taken into the tube from the

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tail of the rat with aid from an attached tuberculin syringe. The blood was diluted in saline on a slide, and the entire sample examined for microfilariae. Successive triple counts from infected rats were analyzed by the method of Scott (1938); a coefficient of variation of 4.4% was found, and hence, the method was considered consistent enough for these experiments. Counts of microfilariae were performed in the same manner for all rats, and at the same time of the day. The statistical methods used were those of Snedecor (1946). Statistical differences between groups were regarded as significant when the probability was 5% or less.

EXPERIMENTAL PROCEDURES AND RESULTS

Skin Infections. Known numbers of infective larvae were introduced subcutaneously into rats, followed by necropsies at intervals of 7 to 45 days to note worm recovery in the pleural cavities. Approximately 2,000 larvae were divided among 11 white rats; the percentages of worms recovered ranged from 0 to 9, with an average of 2%. Similar infections of 14 cotton rats with 3,000 larvae resulted in a range of percentages of 13 to 70, with an average of 42%. An analysis of variance indicated that the percentages of worms recovered in either species of rat did not vary significantly with the longevity of the infection. In white rats a significant number of larvae apparently remain in the tissues and do not complete their migration from the skin to the pleural cavity. In cotton rats, the migration is largely completed within 7 days after infection with approximately one-half of the larvae completing migration.

Results of Transfer Infections. In this experiment, cotton rats were infected subcutaneously; the worms were allowed to complete migration to the pleural cavities, and to grow there for varying periods of time before transfer to the peritoneal cavities of white and cotton rats for further periods of growth. One can thus observe the survival of worms in white rats whose resistance to larval migration has been bypassed. Although this parasite normally selects the pleural cavity for development, a few worms are found in the peritoneal cavity in either natural or experimental cotton rat infections where they develop as do worms in the pleural cavity (Scott and Macdonald, 1953).

Young adult worms were transferred from a donor cotton rat after 26 days' growth, into a white rat and a cotton rat for 9 days' further growth (Table I, group 1). Results were analyzed by use of the one-tailed "t" test. Male worms survived and grew equally well in the two species of rats. Female worms survived

TABLE I.—*Survival, growth and development of worms transferred after: (1) 26 days in a cotton rat, to white (WR) and cotton rats (CR) for 9 days' parallel growth, (2) transferred after 17 days for 9 days, (3) transferred after 7 days for 28 days.*

		Worms transferred	Per cent recovered	Mean length of worms in mm (number measured)		Percent worms in final molt or stage	
				Males	Females	Males	Females
(1)	WR	38	39	12.7 ± 1.0 (8)	15.7* ± 2.5 (5)	100	100
	CR	38	63	14.9 ± 0.5 (18)	27.5 ± 3.4 (6)	100	100
(2)	WR	47	77	10.1 ± 0.4 (15)	11.7 ± 0.5 (21)	100	95
	CR	47	60	8.1 ± 1.3 (10)	10.7 ± 0.4 (18)	80	67
	WR	38	66	8.7 ± 0.5 (11)	9.4 ± 0.5 (11)	91	64
	CR	38	61	8.6 ± 0.8 (6)	10.6 ± 0.3 (17)	83	47
	WR	53	51	8.1 ± 0.5 (14)	10.3 ± 0.2 (12)	71	25
	WR	53	49	8.3 ± 1.1 (12)	9.6 ± 0.4 (10)	92	60
	CR	36	42	8.9 ± 0.8 (6)	10.4 ± 0.5 (7)	100	57
(3)	WR	40	40	12.5 ± 0.6 (8)	15.7* ± 3.0 (7)	100	100
	CR	37	49	15.0 ± 0.6 (14)	31.3 ± 3.6 (4)	100	100
	WR	39	10	14.4 ± 0.6 (4)			
	CR	35	9	15.6 ± 0.8 (3)			
	WR	68	21	14.9 ± 2.8 (5)	37.5 ± 2.3 (8)	100	100
	CR	69	81	16.9 ± 0.2 (34)	45.6 ± 1.0 (34)	100	100

* Difference statistically significant at the 5% level.

equally well in either rat, but grew significantly less in the white rat. Worms transferred to the cotton rat grew as well as control worms allowed to grow the entire 35 days in a cotton rat (mean lengths of male and female control worms: 15.1 and 23.4 mm, respectively).

Fourth-stage larvae were transferred from donor cotton rats after 17 days' growth into white and cotton rats for 9 days' further development (Table I, group 2). No significant differences between worms from white rats and cotton rats were found with respect to percentage recovery, mean length or percentage in final moult or adult stage. In all these respects transferred worms developed as well as control worms allowed to remain the entire 26 days in a cotton rat (mean lengths of male and female control worms: 6.8 and 8.6 mm, respectively).

Third stage larvae were transferred from donor cotton rats after migration, i.e., after 7 days' infection, to white rats and cotton rats for an additional 28 days' growth (Table I, group 3). These larvae were able to survive and develop into young adults equally well in either species of rat. Worms grew as well in either host with the exception of 1 group of female worms. The mean lengths of male and female worms transferred into cotton rats did not differ significantly as compared to control worms allowed to remain the entire 35 days in 3 cotton rats (mean lengths of male and female control worms: 15.4 and 32.4 mm, respectively).

In group 1 and 2 transfers, the worms recovered from white rats were free of encapsulation by host cells. In group 3 transfers, 11% of worms recovered from white rats showed definite signs of encapsulation. By contrast, worms transferred into cotton rats were free of encapsulation at autopsy.

Production of Microfilariae. The previous experiments have shown that transferred worms survived approximately as well in white rats as in cotton rats for periods of 9 to 28 days. This experiment tested the ability of transferred worms to survive and develop beyond the young adult stage and to produce microfilariae (mf). Three donor cotton rats were infected subcutaneously with infective larvae, and were necropsied after 7, 17 and 26 days of infection. The worms were transferred in approximately equal numbers (comparable to those in Table I) into the peritoneal cavities of 9 white rats. Three cotton rats and 3 white rats were infected subcutaneously as controls (65, 75, 125; and 125, 190, 150 larvae, respectively). White rats infected by transfer were necropsied when microfilarial counts dropped below 1 per cu mm. Control rats were necropsied at approximately the same time.

Figure 1 shows, as 3-point moving averages, the results of microfilarial counts from white rats infected by transfer. All rats exhibited a microfilaremia during the infection, although 1 rat in the 7-day group was positive for only a single count (less than 1 mf per cu mm on the 75th day). In general, microfilariae were not detected in the blood of white rats as early as in cotton rat controls (50th and 55th days). Worms transferred after 7 days' growth were less able to survive and produce microfilariae than were worms allowed the longer periods of growth in the cotton rat, i.e., 17 and 26 days.

White rats infected subcutaneously did not exhibit a microfilaremia at any time. Cotton rats, similarly infected, developed high levels of microfilaremia which began on the 50th or 55th day and increased rapidly to levels of 400 to 1500 mf per cu mm after 3–4 months of infection. One cotton rat, however, showed a sudden drop in mf per cu mm after the 65th day and became negative on the 80th day. No explanation is presently available for this occurrence, since both sexes of the adult worms were present and alive at autopsy.

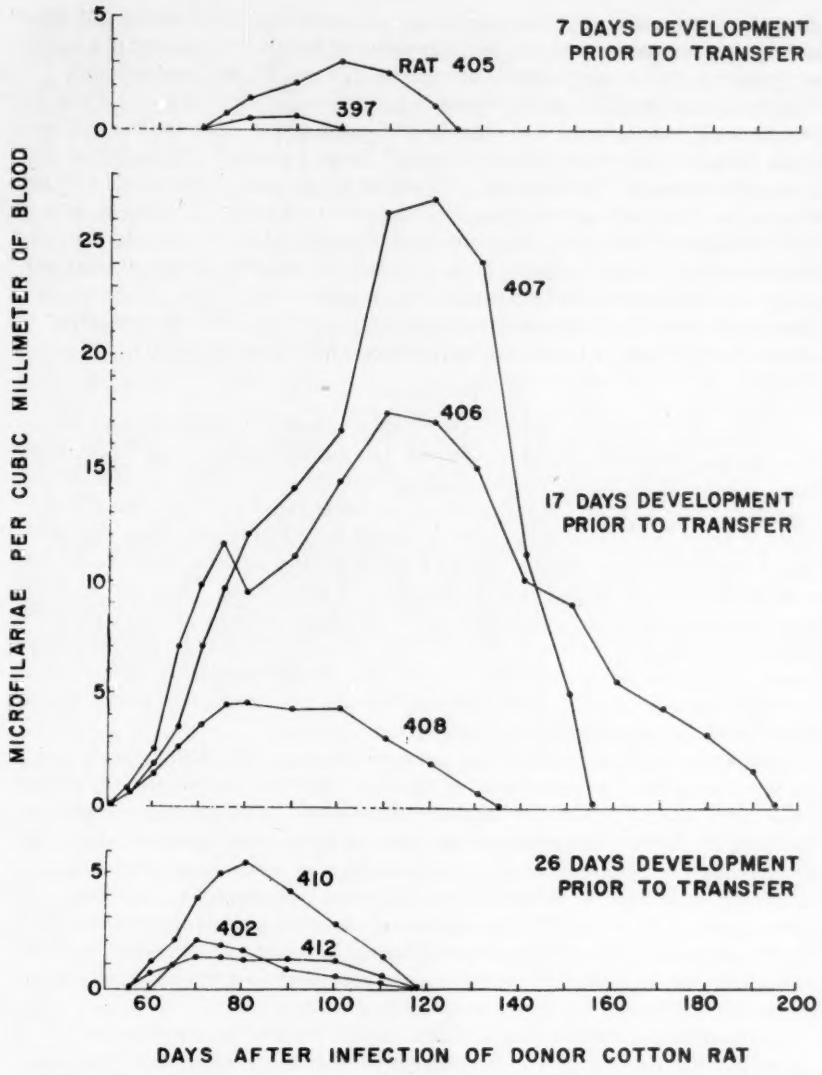

FIGURE 1. Microfilarial counts from white rats infected by transfer with worms which had developed for 7, 17, or 26 days in the cotton rat.

At necropsy, white rats which had exhibited the higher microfilarial levels were also found to harbor the greater numbers of worms. Less than 1% of the worms transferred after 7 days were present at necropsy. Worms transferred after 17 and 26 days showed survival rates of 11% and 10%, respectively. Of the 34 surviving worms in the 9 white rats infected by transfer, all 6 female worms and 4 of the 28 male worms were encapsulated by host cells. White rat controls were negative at necropsy. Cotton rat controls harbored 30% of the original dose; 1 of these worms,

a male, was encapsulated. The mean length of transferred male worms was equal to that in cotton rat controls (21 mm); transferred female worms were not significantly shorter than those in cotton rat controls (76 and 88 mm, respectively).

Development in Mites of Microfilariae from Transferred Worms. White rat 406 (Figure 1) was exposed to a colony of uninfected mites. After feeding and incubation, the mites were dissected and third-stage larvae recovered. These larvae were given subcutaneously to 2 white rats (75 and 48 larvae) and 2 cotton rats (74 and 58 larvae). These rats were necropsied 35 days after infection. The results showed that microfilariae produced in the white rat by transferred worms develop normally in the mite to the infective stage. This conclusion is supported by the average percentage of these larvae completing migration in cotton rats (42%). In white rats, these larvae showed no increased adaptation for survival, since the percentage of worms recovered was as low as that in previous infections of white rats (2%).

DISCUSSION

It seems clear that old white rats are highly resistant to a subcutaneous infection of *L. carinii*. The early period of infection, i.e., during the migration phase of the larvae, appears to be the most critical in the survival of the parasite. Somewhere between the skin and the pleural cavity of the white rat, an environment exists or develops which prevents the majority of larvae from completing their migration. Since this resistance was effective within a few days after infection, it does not seem probable to identify it as acquired, i.e., the result of antibodies. The positive results of the transfer experiment support the conclusion that resistance to migration is the most critical phase in the survival of this parasite in white rats. Late third-stage larvae, which have apparently lost the need for further migration, survived and developed approximately as well following transfer into white rats as did similar worms in cotton rat controls.

Admittedly, resistance in the white rat does not cease after the migration period has been bypassed. A lower level of resistance persists against the transferred worms with stunting and earlier death of the parasite. In shorter periods of infection (9 to 28 days) transferred worms tended to grow more slowly in white rats, although this difference was usually not significant. In infections of several months' duration (experiment on microfilariae) the worms recovered at necropsy were approximately equal in length to those in cotton rat infections. It appears, therefore, that if transferred worms can survive long enough in white rats, they can eventually reach adult size, in spite of a slower growth rate. Most worms, however, are destroyed in the white rat before reaching full adult size.

An interesting correlation can be made between the decreased survival of worms and the increased percentages of encapsulation in the longer infections in white rats. Many of the transferred worms recovered from white rats were encapsulated, as compared to the single encapsulated worm recovered from cotton rats. Encapsulation was noted when transferred worms were allowed more than 9 days' development in the white rat: 11% after 28 days' infection, and 28% after several months' infection. Admittedly, a certain number of worms may be damaged during the manipulation necessary for transfer. For example, when young adult worms, 26 days old, were transferred into the white rat for 9 days' further development, approximately 50% of these worms were recovered (Table I). Since no encapsulated worms or nodules were found at autopsy, about 50% of the transferred worms were completely

destroyed within the 9 days. Survival of worms was similarly reduced in all transfers and did not differ significantly between species of rat. It is concluded, therefore, that some damage to worms does occur in the transfer process. Considering that encapsulation and destruction of an adult worm can occur within 9 days, it seems that factors other than damage from transfer are needed to explain the encapsulation of worms in periods of infection greater than 9 days, i. e., 28 days to several months. In this connection Chandler (1936) has shown that the gradual disintegration of *Nippostrongylus muris,* which were transferred into the peritoneal cavities of rats, stimulated a low level of immunity in the rat to a challenging infection. Macdonald and Scott (1953) reported that a low level of immunity develops in the cotton rat after introducing fresh or dried adult *L. carinii* into the peritoneal cavity.

Another possibility may be an innate superiority of white rats to mobilize a cellular reaction to transferred worms, or migrating larvae, as compared to that in cotton rats. Bloom et al (1950) have postulated a similar hypothesis for the resistance of white rats to tuberculosis as compared to that of rabbits. They noted that the mononuclear phagocytic response of the rat to an irritant in the peritoneal cavity was significantly greater than that for rabbits. Lindquist (1950) in studies with *Nippostrongylus muris* in white rats and cotton rats, suggested that larvae entering the tissues of an abnormal host are prevented from a normal migration by a cellular reaction which leads to the encapsulation and death of the invading larvae. However, he (1952) was not able to find this difference in the cellular response of these same hosts to an initial infection of *Ancylostoma caninum* larvae.

Studies will be reported elsewhere on the cellular responses of white and cotton rats to this infection.

SUMMARY

One-year-old white rats are highly resistant to an infection with *Litomosoides carinii* produced by a subcutaneous introduction of known numbers of infective larvae. In infections of 7 to 45 days, an average of only 2% of these larvae were able to complete migration from the skin to the pleural (or peritoneal) cavity. In similar cotton rat infections an average of 42% completed migration. The resistance of white rats to migrating larvae was bypassed by allowing migration to occur in the normal host, and transferring late-third-stage larvae (or fourth- and fifth-stage worms) into the peritoneal cavities of white rats. Transferred worms survived, developed, and grew for periods of 9 to 28 days approximately as well in white rats as in cotton rats, although there was some evidence of stunting of female worms in white rats.

In longer periods of infection (several months) transferred worms in white rats attained sexual maturity at about the same time as in cotton rat infections, and produced microfilariae which developed normally in the mite into infective larvae. These larvae, however, showed no adaptation for increased survival in white rats. The survival of transferred worms (especially females) in these longer periods of infection was markedly reduced in white rats as compared to cotton rat infections. Encapsulation of transferred worms was not apparent in short infections of white rats (9 days), but was observed commonly (especially for female worms) as the period of infection was increased (28 days to several months). Encapsulation was rare in control cotton rat infections.

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STRAIN DIFFERENCES OF *TOXOPLASMA GONDII*

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Experiments with *Toxoplasma gondii* in tissue culture have revealed properties associated with the differences in virulence of various strains (Kaufman, Remington and Jacobs, 1958). The RH strain, which rapidly kills mice, destroyed monkey kidney and human amnion monolayer cultures in 4–5 days, invading approximately 5.1 times as many cells and multiplying 2.25× as fast as an equal inoculum of the 113-CE strain, which is relatively low in virulence for mice and caused only erratic lysis of the cultures. The S5 strain, of intermediate virulence for mice, destroyed monkey kidney cultures in 10 days and was intermediate in its invasiveness and rate of multiplication. Further experiments concerning virulence and the prolonged infection of tissue cultures by slowly multiplying organisms, are reported below. The details of experimental procedures are as already reported by Kaufman, Remington and Jacobs (1958).

In an attempt to equate the initial tissue infection by the RH strain and 113-CE strain, 1 million 113-CE organisms were inoculated into each of 20 monkey kidney monolayer tubes and 200,000 RH organisms were inoculated into 20 similar tubes. The organisms were permitted 3 hours to attach before the inocula were washed off. The tubes were incubated in stationary racks, fixed and stained after 24½ hours, before lysis of cells had occurred; the numbers of intracellular organisms were counted and are tabulated in Table I. Marked strain differences in the rate of multi-

TABLE I.—*Comparison of the number of parasitized cells and of parasites per cell in rolling and standing monkey kidney tissue cultures infected with 2 strains of* Toxoplasma.*

Standing cultures				Rolling cultures			
113-CE		RH		113-CE		RH	
% cells infected	No. of Toxoplasmas per cell	% cells infected	No. of Toxoplasmas per cell	% cells infected	No. of Toxoplasmas per cell	% cells infected	No. of Toxoplasmas per cell
21	3.3	10	12.1	8	2.8	2	11.5
15	2.9	16	9.7	5	2.4	4	9.0
15	3.3	9	15.6	5	1.6	1	6.0
14	4.0	14	9.8	5	3.8	6	10.2
Ave. 16.3	3.4	12.3	11.8	5.8	2.6	3.25	9.2

The inoculum of 113-CE parasites was 1×10^6, 5 times the inoculum of RH parasites (2×10^5) to compensate for the difference in invasiveness in the strains.

plication can be seen. There was an average of 3.5 times as many RH strain organisms within each cell as of strain 113-CE. Although more cells were initially

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exposed to the very large inoculum of 113-CE organisms, the more virulent RH organisms destroyed the cultures more rapidly and consistently (Table II).

TABLE II.—*Lysis of monkey kidney tissue cultures infected with 2 strains of* T. gondii.

Strain	Number of Tubes	Time for Destruction of Cell Sheet
RH	12	4 days
113-CE	2	12 days
	10	No lysis in 26 days

In order to investigate the effects of the time of attachment and penetration, 4 tubes of each strain were treated as above, except that both during the attachment of the organisms and during reincubation after the 3 hours permitted for attachment the tubes were kept in a roller drum (1/5 RPM). A comparison of the cellular invasion occurring in stationary tubes as opposed to revolving tubes (Table I) reveals much less infection in the revolving tubes. This suggests that more prolonged contact between the organisms and the tissue is necessary for infection.

This method of testing the rate of invasion by *Toxoplasma* in rolling and stationary cultures is obviously more sensitive than that previously used in this laboratory by Cook and Jacobs (1958). In the latter study, lysis time of cultures, after various intervals of exposure to parasites, was the principal criterion. Because of the rapid multiplication of RH parasites, differences in initial percentages of infected cells were obscured after sufficient time of incubation, and lysis of the cultures occurred within a narrow time range.

Several of the cultures infected with strain 113-CE were not destroyed, and "chronic" or prolonged infection of the tissue culture seemed to occur. A similar phenomenon was observed in 4 monkey kidney tissue culture tubes infected with organisms of another avirulent strain, 721. Although after 26 days some cell sheets had ragged edges and slightly granular-appearing cells, many cell sheets were completely intact and, under low power observation, appeared indistinguishable from uninfected control tubes. Four of the 113-CE- and 2 of the 721-infected tissue cultures were fixed and stained. On histologic examination the proportion of infected cells was very small (less than 1%) and even the number of organisms per cell was small. When replicate cultures were inoculated into mice, live organisms were recovered. In culture, therefore, the organisms were alive, but apparently multiplied so slowly and caused such little cell destruction that cellular regeneration was sufficient to maintain the integrity of the tissue culture and mask any cell destruction. This suggests the possibility that chronic infection in vivo may be maintained by relatively dormant organisms in equilibrium with the host tissues. The tough, digestion-resistant hard-walled cysts may be more important for the spread of infection to new hosts rather than in the production of chronic disease. In addition, Daraprim (a folic acid antagonist) and sulfadiazine (a paraminobenzoic acid antagonist) may be expected to be less effective against slowly dividing organisms such as these, since metabolic antagonists are known to be more effective against rapidly dividing organisms.

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THE SIZE OF THE OOCYSTS OF *EIMERIA LABBEANA*

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Size and shape are the features of oocysts most often described, and variation in size during the course of infection has been noted by several authors. Fish (1931), working with *Eimeria tenella,* and Becker, Zimmermann, and Pattillo (1955), working with *E. brunetti,* found that oocysts late in the patent period tended to be significantly larger than those measured early in the infection. Jones (1932) observed significant variations in the size of *E. acervulina* oocysts from day to day in the same bird and in different birds on the same day. Oocysts were markedly larger when an infection had been produced by a single oocyst than when a massive dose had been given. The smaller size was thought to be due perhaps to a crowding effect. On the other hand, Doran and Jahn (1952) reported that no significant difference in oocyst length appeared during infections of *E. mohavensis* in the kangaroo rat.

Oocysts of *E. labbeana,* a coccidian parasite of the domestic pigeon (*Columba livia*), have been measured by a number of investigators including Nieschulz (1935), Bondois (1936), and Morini (1950). However, it appears that none of their measurements were recorded with reference to the time of infection. Therefore, if the dimensions of oocysts differ at different times during an infection, such measurements might be meaningless, as Fish (1931) and others have pointed out.

In the present investigation the author made numerous measurements of the oocyst size in *E. labbeana* at various times during the patent period. Putting all the measurements together resulted in the determination of mean dimensions of 19.1 × 17.4 microns, with a range of 14.5–24.1 microns in length and 12.9–22.5 microns in width. In 13 of more than 300 experimentally produced infections the average dimensions of oocysts were appreciably smaller than the overall average. In all 13 cases the smaller oocysts appeared early in the infection. As the infection progressed, the mean dimensions of the oocysts in 10 of the 13 cases increased until on the final days of the patent period the means approximated the overall average (Table I).

Oocysts of infections #5 and #9 remained smaller than average even late in the infection. Sporulated oocysts from these two infections, collected late in the patent period, were used to infect 2 coccida-free birds, and when oocysts appeared, they were measured early and late in the patent period. As Table II shows, the mean dimensions increased, until late in the patent period they approximated the overall average.

The small size of oocysts did not seem to be related to the degree of infection. Small oocysts appeared during both light and heavy infections. The number of oocysts in an initial infecting dose showed no correlation with oocyst size.

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TABLE I.—*Mean dimensions of oocysts early and late in the patent period of 13 infections in which the oocysts were initially small. ML = mean length (microns) MW = mean width (microns).*

Infection Number	First Day		Fifteenth Day	
	ML	MW	ML	MW
1	16.7	15.2	19.3	17.6
2	15.5	14.1	19.0	17.4
3	15.6	14.1	19.3	17.6
4	16.2	15.0	19.1	17.5
5	18.0	16.6	18.1	16.6
6	16.7	14.9	20.0	18.1
7	17.5	16.0	19.0	17.4
8	15.5	14.0	19.2*	17.5
9	17.4	16.1	17.6	16.2
10	16.2	14.9	18.9	17.3
11	16.7	15.2	19.0	17.3
12	18.1	16.8	19.1	17.7
13	15.3	14.0	18.1	15.9

* 13th day.

TABLE II.—*Mean dimensions of oocysts from infections produced by small oocysts.*

Infection Number	First Day		Fifteenth Day	
	ML	MW	ML	MW
5a	18.5	16.9	19.0	17.3
9a	17.8	16.5	19.1	17.5

Although it is apparently impossible to predict the production of small oocysts, they seem always to appear at the very beginning of the patent period.

SUMMARY

The mean dimensions of *E. labbeana* oocysts collected early and late in the patent period remained relatively constant during the course of infection in most cases. When small oocysts appeared, they occurred early in an infection, and the mean dimensions of oocysts measured late in the patent period of the same infection were usually larger. The production of small oocysts could not be explained by a crowding effect.

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THE EFFECTS OF SOME CHEMICAL AND PHYSICAL AGENTS ON THE OOCYSTS OF THE PIGEON COCCIDIUM, *EIMERIA LABBEANA* (PINTO, 1928)

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Because the oocysts of a coccidian life cycle are easily collected and cultured, they have been investigated more thoroughly than other stages of coccidia. Studies of the effects of various agents on oocysts are aimed at discovering (1) if the agent will prevent sporulation, and (2) if it will kill the oocysts. Either result would prevent oocysts from infecting, but in the former case sporulation might occur if the agent were removed.

A number of investigations of the resistance of oocysts have dealt with the coccidian parasites of poultry (Beach & Corl, 1925; Becker & Crouch, 1931; Fish, 1931; Herrick, 1935; Chang, 1937; Ellis, 1938; Brotherston, 1948; and Edgar, 1954) Most other coccidia, including *Eimeria labbeana*, have been neglected. Bondois (1936) and Morini (1950) dealt with resistance briefly as relatively minor parts of their larger studies on *E. labbeana*. Chakravarty and Kar (1946) investigated the effect of temperature, only, on *E. labbeana* oocysts.

MATERIALS AND METHODS

Feces from domestic pigeons (*Columba livia*) heavily infected with *E. labbeana* were collected and transferred to Petri dishes or watch glasses where they were spread out in a test or control solution which was of sufficient quantity to cover them. When it was necessary to concentrate oocysts for counting, the technique used was the zinc sulfate method, designated Technic # 8 by Faust et al (1939).

A solution of 2% $K_2Cr_2O_7$ was found to give consistently high sporulation at a room temperature range of 20–26° C, and this solution was utilized as a control medium. This room temperature range (20–26° C) was used during all the tests of oocyst resistance except for those dealing with the effects of temperature, where the 20–26° C range was utilized as the control. Test substances used in the present investigation were distilled water, sodium chloride solution, hydrochloric and acetic acids, sodium bicarbonate, and formalin. The salt, acids, base, and formalin were all tried at more than one concentration.

The effects of moderately high and low temperatures were also tested. Feces in 2% $K_2Cr_2O_7$ solution were placed in refrigerators or ovens for varying lengths of time. Resistance to desiccation was tested by placing fresh feces containing oocysts on filter paper in a room where relative humidity did not go higher than 46%. To check the effect of light on sporulation, oocysts were placed in a very shallow layer of $K_2Cr_2O_7$ and exposed to light for varying lengths of time.

Anaerobic sporulation was tested in a Gradwohl anaerobic jar. The oocysts were placed in 2% $K_2Cr_2O_7$ in a 30-ml test tube. After the jar was closed, gas was added for 15 minutes, and the jar was immediately sealed. All oocysts were checked immediately upon removal from the Gradwohl jar.

As a test of viability of unsporulated forms, oocysts were returned to control conditions for as long as 10 days and examined for signs of sporulation. Sporulated oocysts were fed to coccidia-free pigeons to check their ability to infect.

For determination of the percentage of sporulation that occurred under control and test conditions 100 oocysts were counted at 440×. A sporulated oocyst was considered to be one in

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which the sporocysts appeared fully formed and contained sporozoites. Since it would have taken a great deal of time to determine the degree of development of the sporozoites, their stage of maturity was not checked.

RESULTS

Sporulation amounting to 100% was never seen either in control or test media at various temperatures. This observation differs from that of Chakravarty and Kar (1946) who reported 100% sporulation of *E. labbeana* oocysts within 96 hours. Their medium was 2.5% $K_2Cr_2O_7$ and the temperature was 22.2° C. In the present investigation the highest observed sporulation was 97%, which was unusually high. Under control conditions, i.e., 20–26° C in 2% $K_2Cr_2O_7$, the range of the mean percentage of sporulation after 4 days was 83–88%.

It is of interest to note that oocysts were able to infect coccidia-free pigeons after 400 days at control conditions.

Distilled water proved to be a poor medium for sporulation, as Bondois (1936) found for *E. labbeana* and other authors have noted for a number of other coccidia. The few oocysts that sporulated in water remained viable for periods up to 30 days, since after the addition of $K_2Cr_2O_7$ they sporulated and were able to infect coccidia-free birds. After being in water for longer than 30 days, however, none of the oocysts remained alive.

Sporulation of oocysts proceeded rapidly in 10% and 15% NaCl solutions but was reduced in 20% NaCl. After remaining in 10% and 15% NaCl solutions for 10 days, oocysts were still able to infect coccidia-free pigeons, even although such oocysts were distorted and appeared dead. Some oocysts remained viable after 3 days in 20% NaCl, but all were dead after 4 days.

Sporulation of oocysts was slightly reduced in 2% and 5% HCl, but not at all in 5% CH_3COOH. A 2% solution of $NaHCO_3$ reduced sporulation greatly, and no sporulation occurred in 5% $NaHCO_3$. Oocysts that had been in these solutions, with the exception of 5% $NaHCO_3$, for 10 days were still infective. A 10-day 5% $NaHCO_3$ oocyst sample was placed in 2% $K_2Cr_2O_7$ and no oocysts sporulated.

Although Pérard (1925) reported that oocysts from rabbits segmented in 5% formalin and Morini (1950) found that *E. labbeana* oocysts sporulated in 2.5% formalin, in the present study none sporulated in 2% and 5% formalin. Sporulation occurred normally, however, in 1% formalin.

Oocysts subjected to temperatures of 0–2.5° C and 3–4° C did not sporulate while at the low temperatures. Upon return to room temperature after 60 days at 3–4° C, some oocysts sporulated, although sporulation was somewhat reduced. None of the oocysts that had been at 0–2.5° C for 60 days sporulated upon return to room temperature. Thirty-day samples showed segmentation.

Sporulation of oocysts was tested at 30, 32, 35, 37, 38, and 40° C. At 30 and 32° C, sporulation was reduced slightly. At 35° C, sporulation occurred in only 3 of 500 oocysts observed. At 37, 38, and 40° C, no sporulation was seen. No sporulation occurred when oocysts were returned to room temperature after 3 days at 35° C and 2 days at 37° C. These results correspond closely with those of Bondois (1936) and Chakravarty and Kar (1946), except that they did not utilize as many different temperatures.

Contrary to Morini's (1950) findings, high temperatures did not produce more atypical oocysts than normal, although at temperatures above 35° C the protoplast disintegrated rather rapidly.

Severe distortion resulting from drying made it impossible to determine the percentage of sporulation during the test dealing with the effect of desiccation on oocysts. Therefore, the ability to infect was investigated, rather than the ability to sporulate. After drying at room temperature for 3 days oocysts were still infective, even although greatly distorted. After 4 days, however, the oocysts were unable to infect coccidia-free pigeons even after being placed in $K_2Cr_2O_7$, and were presumably dead.

Fresh oocysts were exposed to light for varying lengths of time. No effect was noted on the percentage of sporulation or on the sporulation time. This result agrees with those of Pérard (1925) and of others who worked with a number of coccidia.

Numerous authors have stated that oxygen is necessary for sporulation of oocysts, and yet apparently none have attempted to culture oocysts anaerobically. On the other hand, Goodrich (1944) felt that oxygen was not essential for sporulation and based her opinion on the fact that a reducing agent may be used to ripen oocysts.

In the present investigation, oocysts were kept under anaerobic conditions for 3, 5, 10, 15, and 30 days. In each case sporulation took place but was reduced greatly. After return to air, oocysts that had been in a Gradwohl jar 10 days and longer showed no more sporulation. Three- and 5-day samples sporulated rapidly after return to air until their percentage of sporulation was about average. Although the percentage of sporulation of 10-, 15-, and 30-day samples did not rise after return to air, the oocysts were able to infect pigeons.

DISCUSSION

The percentage of sporulation of *E. labbeana* oocysts in water at optimal temperature was unexpectedly small. It is probable that bacteria prevent more sporulation under such conditions, since the addition of a bactericidal prevent more potassium dichromate, to water containing oocysts results in sporulation commencing immediately. This inability of oocysts to sporulate well in the presence of bacteria may well account for the fact that most coccidian infections under natural conditions are light. Although large numbers of oocysts may be ingested, relatively few are sporulated forms, capable of infecting the avian hosts.

It was found that 1% formalin could be used as a sporulation medium for *E. labbeana* as Hall (1933) had utilized it for *Isospora lacazei.* However the value of formalin as a sporulation medium is limited since the oocysts lose their ability to infect coccidia-free pigeons after being in formalin for 20 days.

The oocysts that were tested in the present study were more resistant to the effect of NaCl than those observed by Bondois (1936). Whereas he reported that *E. pfeifferi* (*labbeana*) oocysts were all killed within 24 hours in a 15% NaCl solution, in the present investigation oocysts were still alive and able to infect pigeons after 10 days in 15% NaCl and after 3 days in 20% NaCl. A possible explanation for this difference in results may be that Bondois apparently relied solely on the appearance of the oocysts in determining viability. He reported no attempts to infect pigeons with the oocysts that had been in NaCl. It was shown in the present study that even severely distorted oocysts may be infective and can not be considered dead unless unable to infect coccidia-free birds.

Oocysts dried in air must likewise be tested for infective ability before any assumption concerning their viability may be made. Oocysts dried at room temperature for as long as 3 days were found to be severely distorted but still infective although Bondois (1936) had reported all oocysts dead after drying at 20° C for 48 hours.

Why oocysts of *E. labbeana* sporulated well in dilute hydrochloric and acetic acids and poorly in equal concentrations of sodium bicarbonate is not known. Perhaps this difference in sporulation is related to the low pH of the pigeon intestine.

The observation that the oocysts of *E. labbeana* did not sporulate at moderately low temperatures agrees with what is known of many species of coccidia. The ability of oocysts to sporulate after return to room temperature following exposure to cold is not as well known. Chakravarty and Kar (1946) found that *E. pfeifferi* (*labbeana*) oocysts did not sporulate within 24 hours after return to room temperature following their exposure to a temperature of 4° C for 168 hours. On the other hand oocysts utilized in the present investigation showed reduced ability to sporulate within 24 hours at room temperature after exposure to a temperature of 3–4° C for 60 days. Sporulation also occurred following exposure for 30 days to a temperature of 0–2.5° C. When left at room temperature longer than 24 hours after exposure to cold many more oocysts sporulated. From these results it appears that the oocysts of this coccidium may be much more resistant to cold than has been reported previously.

That so few oocysts sporulated at 35° C is surprising. Since no sporulation occurred at 37° C or at any higher temperatures, it may be assumed that the high body temperature of the host prevents sporulation within the bird. The body temperature of the pigeon does not go lower than approximately 40° C.

The results of testing oocysts anaerobically indicate that oxygen may not be necessary for sporulation. Reduced sporulation apparently can occur without oxygen and some oocysts may remain alive and infective after remaining in a Gradwohl jar for as long as 30 days. This evidence indicates that lack of oxygen is not a factor in preventing sporulation in the host.

SUMMARY

The effects of NaCl, HCl and CH_3COOH, $NaHCO_3$, formalin, moderately high and low temperatures, light, and lack of oxygen on the viability and ability of *E. labbeana* oocysts to sporulate were investigated. The oocysts were found to be considerably more resistant to NaCl concentrations, moderately low temperatures, and drying, and less resistant to formalin than some results in the literature would indicate. More segmentation occurred in dilute acid than in dilute alkali. Little sporulation occurred at 35° C and none at 37° C or higher. The amount of light to which the oocysts were exposed had no effect on sporulation. Reduced segmentation occurred under anaerobic conditions, and the oocysts remained infective after 30 days without oxygen.

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ERRATUM

The writers wish to call attention to the regrettable error in nomenclature that appeared in our recent paper (1957, J. Parasit. **63** (6): 649–655). The subfamily name Anhingofilariinae is to be replaced by Wymaniinae n. n.—EVERETT E. WEHR AND JOSEPH C. HWANG.

THE DESCRIPTION OF *HAEMOGREGARINA BOYLI* N. SP. FROM THE YELLOW-LEGGED FROG, *RANA BOYLI BOYLI**

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The first haemogregarines to be found in the Salientia of North America were noted by Langmann (1899) from many "frogs". Stebbins (1904) described *Haemogregarina catesbianae* from *Rana catesbeiana* and (1905) *Karyolysus clamatae* in *R. clamitans* and *R. pipiens;* Kudo (1922) presumably found the same parasites in *R. pipiens* and *R. clamitans.* Sanders (1928) noted *Lankesterella* in *R. clamitans* and *R. catesbeiana* and, in addition, she found *Karyolysus* in *R. clamitans.* Brandt (1936) found haemogregarines (*Karyolysus* or *Lankesterella*) in *R. catesbeiana* and *R. sphenocephala.* Fantham et al (1942) described *Lankesterella canadensis* from *R. catesbeiana* and *Dactylosoma sylvatica* from *R. sylvatica;* they also found haemogregarines in *R. pipiens, R. catesbeiana* and *Bufo americanus.* Fowler (1946) found haemogregarines in grass frogs and, finally, Bailey (1948) reported a haemogregarine from *R. pipiens.*

During 1957 and 1958 nineteen Yellow-legged frogs, *Rana b. boyli,* were collected from Sutter Creek, Amador County, California. Examination of the peripheral blood revealed the presence of sporozoa in the erythrocytes of 7 of the individuals. A portion of the life cycle in the vertebrate host was determined by a study of blood smears and impressions and sections of the viscera. The parasite is dissimilar to the other described species of anuran haemogregarines and, consequently, it is considered a new species. In addition to the description of the new species, inoculations of infected cells were made into presumably uninfected *Rana b. boyli.*

MATERIALS AND METHODS

Peripheral blood was obtained by clipping one of the digits; thin smears were then stained by 1) Wright's stain, 2) Giemsa's stain, 3) Giemsa's stain, following fixation in Schaudinn's fluid, or 4) Heidenhain's iron haematoxylin.

Organs and tissues that were examined for the presence of developmental stages were the liver, lungs, heart, kidneys, bone marrow and gut wall. Impressions or smears were made of each organ and, following fixation in Schaudinn's fluid, were stained with Giemsa's or haematoxylin. Sections of organs 10 microns in thickness were also stained with Giemsa's or haematoxylin.

Cross inoculations were made into 4 *Rana b. boyli* collected from Sutter Creek. Prior to inoculation the peripheral blood was examined every other day for a period of 2 weeks, and, if haemogregarines were not found, the animal was presumed to be uninfected. One ml infected heart blood, suspended in a like amount of 0.7% saline, was injected into the abdominal vein of 2 of the frogs; the remaining 2 frogs received 2-ml intravenous injections consisting of equal amounts of macerated liver and saline. Following injections, the animals were maintained at 10°C, and thin blood smears were examined every third day for a period of 30 days.

All descriptions, unless otherwise stated, are made from films, impressions and sections which were stained with haematoxylin.

Haemogregarina boyli n. sp.

Diagnosis: Haemogregarines which undergo schizogony in the erythrocytes and erythroblasts of the liver. Mature schizonts divide into 2 merozoites of identical dimensions; merozoites penetrate erythrocytes and develop into sausage-shaped gametocytes which are found in the peripheral blood.

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Host: Rana b. boyli.
Location: Circulatory system.
Locality: Sutter Creek, Amador Co., Calif.

Location of Parasites in the Host

Regardless of the tissues examined, intracellular parasites were found only in erythrocytes and erythroblasts; their positions in the cell were somewhat variable, some were peripheral, others central. In all parasitized cells, except those recently penetrated by merozoites, the host nucleus was displaced, but at no time was the structure atrophied. The cytoplasm surrounding each parasite was also normal except for a clear area around the parasite. Host cells containing recent penetrants were of the same size as unparasitized cells, but the host cells containing mature gametocytes and schizonts were greatly enlarged, often to twice the size of normal cells. Extracellular merozoites were found in all tissues examined.

Parasites of the Peripheral Blood

Two types of intracellular parasites were detected in erythrocytes and erythroblasts of the peripheral blood: trophozoites and gametocytes. Trophozoites (Fig. 1) averaged 10.4 (8.2–17.1) microns in length by 4.1 (3.9–6.4) microns in width. Their shapes varied, the more mature protozoa being sausage-like, while the younger stages were somewhat curved with one end being rounded and the other bluntly pointed. The nuclei of both types were granular and situated more toward one end than the other.

Two types of gametocytes were found but the writer hesitates to designate the sex of either until the invertebrate cycle can be determined. Type A was 12.3 (11.4–15.4) microns in length by 4.9 (4.2–5.6) microns in width. The granular, circular nucleus was central in position and not surrounded by a membrane. The body was a modified dumb-bell shape with the central portion somewhat narrower than the extremities. Type B gametocytes (Fig. 2) were similar to type A; the difference being in the structure of the nucleus: in type B gametocytes the circular granular nucleus contained a large chromatin dot (endosome?) surrounded by a clear area; the chromatin dot was either central or sub-centric.

Extracellular rod-shaped merozoites were rarely found in the peripheral blood (Fig. 3). They were 10.9 (10.0–11.5) microns in length by 3.3 (3.1–4.0) microns in width. The massive nucleus, 3.5 in length by 3.3 in width, was formed of numerous darkly staining granules and rods; no nuclear membrane was seen. The nucleus was usually centrally located. The cytoplasm was homogenous.

Inasmuch as most haemogregarines are described from air-dried, Romanowsky-stained, thin films of the peripheral blood, *H. boyli* should be briefly considered in the same manner. When peripheral blood was stained with Giemsa's the following was seen: Trophozoites and gametocytes were similar in size to those seen in haematoxylin preparations; capsules were also evident. The cytoplasm stained an irregular white and, on occasion, small vacuoles or otherwise unstained areas were seen; polar caps were never seen. Nuclei stained a reddish color but a nuclear membrane was never seen.

Gametocytes could be distinguished from the trophozoites by size and shape and it was possible to differentiate types by the staining character of the nucleus: type A possessed the homogenous nucleus, while type B possessed the nucleus with the large endosome surrounded by a clear area.

Parasites of the Viscera

Stained smears and sections of the viscera and various tissues revealed the following: the red blood cells of the lungs, heart, kidneys, bone marrow and gut wall contained forms similar to those found in the peripheral blood. When liver films were examined, however, it was found that 78% of the erythrocytes and erythroblasts were parasitized; endothelial and hepatic cells were not parasitized. A wide variety of morphological types were encountered in the liver, many of which were not seen in any other location.

Extracellular merozoites, similar to those noted in the peripheral blood, were found in large numbers in liver preparations. The free merozoites penetrated either an erythrocyte or an erythroblast (Fig. 4), but double infections were not seen. Morphologically, the penetrants were identical with the free forms. By an increase in dimensions, these intracellular merozoites developed into trophozoites which were identical with those described from the peripheral blood.

The schizont, which developed from the trophozoite, divided by a transverse constriction; one end was merozoite-shaped while the other extremity was pyriform or rounded (Fig. 5). The rounded or pyriform body assumed the typical merozoite shape either before or shortly after separating from the sister cell (Fig. 6). When schizogony was completed, the host cell contained 2 merozoites of similar dimensions and of a size identical with those found in the plasma. The positions of the paired merozoites was variable (Figs. 7, 8) and the bodies were released singly or both at one time (Fig. 9).

Gametocytes, of the types described from the peripheral blood, were seen within erythrocytes in liver impressions and sections. In addition to the larger types, smaller and intermediate forms of a similar morphology were seen.

Results of Cross Inoculations

Examinations of peripheral blood of recipient animals were always negative, regardless of the material injected, heart blood or liver suspension. Following the 30-day incubation period, each animal was destroyed, and Giemsa-stained dry smears were made of the liver, spleen, kidneys, lungs, heart and bone marrow; haemogregarines were never found.

DISCUSSION

The term "haemogregarine" has been used indiscriminately as a catch-all to which have been assigned many poorly or incompletely known unpigmented, sausage- or spindle-shaped, intraerythrocytic parasites of the peripheral blood. A number of genera have been included under this designation, but, when dealing with the haemogregarines of the anuran Amphibia it seems that only 2 genera are admissible: *Haemogregarina* and *Karyolysus*. In both, gametocytes are found in erythrocytes of the peripheral blood. Schizogony occurs in endothelial cells in the case of *Karyolysus* while in *Haemogregarina* this process takes place in erythrocytes and erythroblasts; in *Haemogregarina*, trophozoites are often found in the peripheral circulation but schizogony can only rarely be demonstrated in this location as this phenomenon commonly takes place within the internal organs.

"Haemogregarines" of the Amphibia have been described by a number of workers, but, with a few exceptions, only the blood stream forms have been studied. With such material it is difficult to distinguish between gametocytes and trophozoites (when they occur). The writer, for example, was able to distinguish, with cer-

tainty, between gametocytes and trophozoites of *H. boyli* only *after* determining the pattern of schizogony in the liver. Hence, when the peripheral blood alone is examined, it may be impossible to determine the genus to which the parasite belongs unless schizogonic stages are found. As a consequence, the generic designations of many of the previously described haemogregarines of the Amphibia must be considered with reservation, at least until the schizogonic portions of their vertebrate cycle become known.

To the writer's knowledge, *Karyolysus clamatae* Stebbins, 1905 and *Haemogregarina catesbianae* Stebbins, 1904 are the only haemogregarines of the Anura for which a portion of the vertebrate cycle has been determined. Stebbins (1905) states that the schizogony of *K. clamatae* occurs in erythrocytes and results in the production of "a number of small ovoid bodies". This type of schizogony is obviously different from that found in *H. boyli* where 2 merozoites are created. Inasmuch as the schizogony of *K. clamatae* occurs in erythrocytes rather than cells of the viscera or the endothelia, it is obvious that this parasite should be relegated to the genus *Haemogregarina.*

Stebbins (1904) described and figured a multiple schizogony for *H. catesbianae* which, of course, differs from that found in *H. boyli.* In addition, the schizont of *H. catesbianae* is described as being produced by a folding over and ultimate coalescing of the arms of an elongate trophozoite; the schizont of *H. boyli* is produced by an increase in size.

The failure to infect unparasitized frogs with injections containing trophozoites, schizonts and merozoites (as well as gametocytes) can not be explained by experimental data; however, several hypotheses might be advanced that may aid in explaining this phenomenon:

1) Both the parasitized and unparasitized frogs were obtained from the same source. It is possible that the supposedly uninfected individuals may have recovered from a previous infection with *H. boyli* and the immunological responses were sufficient to prevent the establishment of a new infection.

2) A host specificity may exist in which the parasite is physiologically dependent upon and restricted to a single individual within the host species. Such a dependence has been shown by Robertson (1912) in the case of trypanosomes from fresh water fish.

3) Failure to transmit *H. boyli* to a new host may indicate that all of the parasites present are sexual or pre-sexual forms, i.e. gametocytes or, in the case of dividing forms, individuals which will yield merozoites capable of developing only into gametocytes. The dividing forms may be similar to final schizogony yielding gametocytes as found by Reichenow (1910) for *H. stepanowi* from the turtle, *Emys orbicularis.*

4) It is conceivable that infective forms are produced only following sexual reproduction in the invertebrate host.

SUMMARY

Haemogregarina boyli is described from the Yellow-legged Frog, *Rana b. boyli.* The peripheral blood contains both intraerythrocytic gametocytes and trophozoites as well as a few free merozoites. Developmental stages are found within the erythrocytes and erythroblasts of the liver: merozoites develop into schizonts which divide into 2 merozoites, each of which reenters the schizogonic cycle. The origin of gametocytes has not been found.

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EXPLANATION OF PLATE

FIGURE 1. Trophozoite.
FIGURE 2. Gametocyte.
FIGURE 3. Merozoites.
FIGURE 4. Merozoite penetrating an erythrocyte.
FIGURE 5. Beginning schizogony.
FIGURE 6. Later schizogony.
FIGURES 7–8. Paired merozoites.
FIGURE 9. Merozoites escaping from red blood cell.

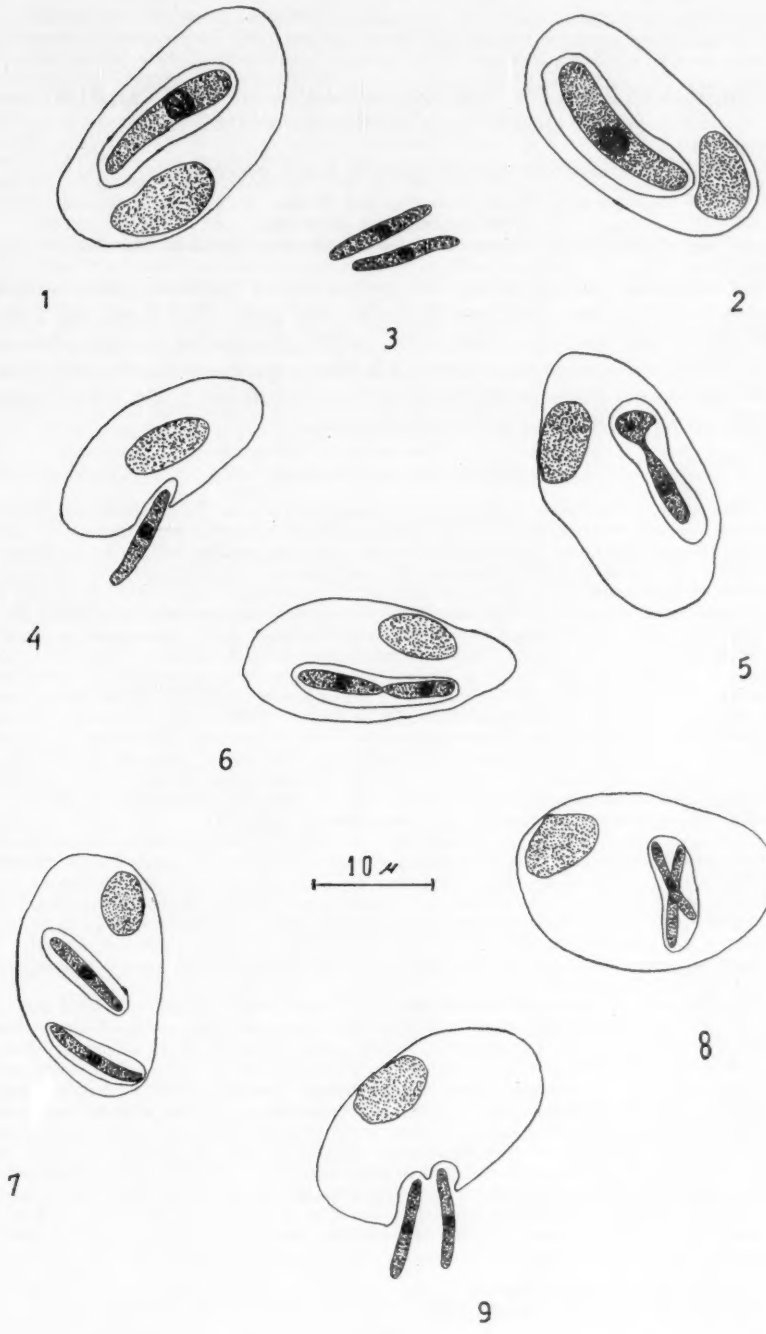
RESEARCH NOTE

EIMERIA BRASILIENSIS TORRES AND RAMOS, 1939, IN WISCONSIN

Oöcysts of *Eimeria brasiliensis* were observed in samples of bovine feces collected to determine the prevalence of bovine coccidiosis in the 71 Wisconsin counties. Each county was sampled twice, in the summer of 1957 and again in the spring, 1958. Oöcysts of *E. brasiliensis* were present in 19 of 390 samples examined in 1957 (4.87%). Two of the 19 samples were submitted to the U.S.D.A. Regional Animal Disease Research Laboratory at Auburn, Alabama, which verified the presence of *E. brasiliensis.* The species was present in 9 samples of 355 (2.54%) examined in the spring of 1958.

E. brasiliensis oöcysts also have been observed in 25 (13.7%) of 182 samples collected from two beef herds in Wisconsin where clinical outbreaks of bovine coccidiosis occurred during 1957–58.

This species of bovine coccidia was first described (1939, Arq. Inst. Pesquisas Agronom. **2**: 79–96) from Brazil by Torres and Ramos. Its presence in dairy cattle in the southeastern U. S. has been reported by Boughton (1945, N. Amer. Vet. **26**: 147–153) and by Davis and Bowman (1952, Proc. 55th U. S. Livestock San. Assoc.). Similarity between *E. brasiliensis* and *E. bohmi* Supperer, 1952, was reviewed by Lee and Armour (1958, J. Parasitol. **44**: 302–304). The present report constitutes a record of the species in northern United States, Wisconsin.—M. R. HASCHE AND A. C. TODD, *University of Wisconsin, Madison.*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OBSERVATIONS ON THE METABOLISM OF THE CULTURE FORM OF *TRYPANOSOMA CONGOLENSE*

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The respiration and the carbohydrate metabolism of the blood stream form of *Trypanosoma congolense* have been studied to some extent (von Brand and Tobie, 1948; Agosin and von Brand, 1954; Ryley, 1956). In contrast, no data whatever are available for its culture forms which, as in other trypanosome species, correspond to the non-infective stages developing in the intermediate host. The present paper is a first attempt to close this gap in our knowledge.

MATERIALS AND METHODS

Cultures of *T. congolense* (Lumu strain), grown according to Tobie (1958) on diphasic blood agar medium were employed for short term aerobic and anaerobic experiments. For each of these, the supernatant fluid from 16 to 24 tubes in which the parasites had grown for 1 week, was pooled. The parasites were concentrated by centrifugation (5 min. at 2700 × G) and resuspended in an appropriate small amount (3 to 4 ml) of supernatant fluid. One-ml amounts of this material were introduced into Warburg flasks of about 6 ml capacity and incubated for 2 hours at 30° C. The rate of oxygen consumption was determined in the conventional way, readings being taken every 15 minutes. When inhibitors were used, the desired concentration was achieved by adding to the flask 0.1 ml of Ringer's solution containing the required amount of the compound. The non-inhibited control received 0.1 ml of Ringer's solution. When cyanide was used, the carbon dioxide was absorbed according to Robbie (1948). The respiratory quotient was determined according to Warburg's direct method with due consideration of the bound carbon dioxide. Anaerobic experiments also were carried out for 2 hours. Anaerobiosis was established by flushing the vessels for 15 minutes with 99.99% pure Linde nitrogen, further purified by passage over hot copper. Both in the aerobic and the anaerobic experiments the number of flagellates was ascertained by counting in a Levy counting chamber.

In all cases where chemical studies were done in addition to the respiratory determinations, the available material was divided into 2 equal parts, one serving for the initial, the other for the final determination. Glucose was determined every time, but only 1 or at most 2 metabolic end-products could be searched for in the limited amount of material available on a given day. In fact, for the succinic acid determinations, pooled material from 3 separate runs had to be used because one day's material usually was not sufficient for this relatively insensitive method. In this case the deproteinized material was kept in a deep freeze until an amount sufficient for ether extraction had accumulated.

In addition to the short-time experiments, longer term aerobic experiments were done as follows. The supernatant fluid of sterile culture tubes which had been incubated for 10 days, was introduced in 20- to 25-ml amounts into sterile, rubber-stoppered, 50-ml Erlenmeyer flasks. This wholly liquid medium was then inoculated with material from a *T. congolense* stock tube. The initial levels of various compounds were determined immediately after inoculation and again after 1 week of incubation at 23° C. Previous experiments with similar flasks had shown that the concentration of the substances under study remained constant within experimental errors when uninoculated flasks were incubated as described above (von Brand, Weinbach, and Tobie, 1955). Such controls were, therefore, not repeated. No counts of organisms were done on these cultures, since it would evidently have been impossible to arrive at a valid average population figure. Instead of calculating the data on the basis of numbers of organisms, as done in the short-time experiments, the production of metabolites was related to the amount of glucose consumed.

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Glucose was determined according to Hagedorn and Jensen (1923), lactic acid according to Barker and Summerson (1941), and keto acids according to Friedemann and Haugen (1943). For total volatile acids the procedure of Bueding (1949) was used and the acids were expressed as acetic acid, since, as shown below, this acid predominates by far. The method of Umbreit, Burris, and Stauffer (1949) was employed for succinic acid with fresh 5% rat liver homogenate serving as source of succinoxidase. Ethyl alcohol was measured by the method of Henry et al (1948). Glycerol was determined by the colorimetric method of Burton (1957) which is based on that of Lambert and Neish (1950). Because the samples contained glucose, correction of the glycerol values became necessary. It was derived from the controls recommended by Ryley (1955).

For identification of the volatile acids, approximately 500 ml of overlay were deproteinized with tungstate and centrifuged. The supernatant fluid was acidified with H_2SO_4 and extracted with ether in a Friedrich extraction apparatus. The ether was evaporated after some distilled water and sodium bicarbonate had been added. The resulting aqueous solution was acidified with H_2SO_4 and steam-distilled, 100 ml distillate being collected. Ten ml were titrated with 0.050N NaOH, using bromophenol blue as indicator. One the basis of this test the remaining 90 ml were neutralized by the addition of 5.0 ml of 0.050N NaOH, without the use of an indicator. The solution was concentrated to about 1 ml by distillation and then brought to dryness over $CaCl_2$ at room temperature. The salts were acidified with 7N H_2SO_4, some anhydrous sodium sulfate was added and the material extracted with about 0.4 ml ether. The ethereal solution was analyzed in 2 portions in a Fisher-Gulf partitioner by Mr. J. E. Kolter.* On the basis of these analyses it can be stated that the volatile acids consisted of more than 99% acetic acid, and less than 1% butyric acid. A trace of propionic acid may also have been present.

RESULTS AND DISCUSSION

The aerobic respiration of the flagellates was fairly sensitive to cyanide and very sensitive to the sulfhydryl inhibitors iodoacetamide and arsenite. It was inhibited only slightly, but significantly, by the tricarboxylic cycle inhibitors, fluoroacetate and malonate (Table I). On the whole, this response is similar to that found in other

TABLE I.—*Influence of inhibitors on the oxygen consumption and motility of the culture form of* Trypanosoma congolense.

Inhibitor	Concentration	Percent inhibition	Motility
KCN	M/2,200	59 ± 3.2 (4)*	little
KCN	M/10,000	35 ± 2.2 (4)	moderate
Iodoacetamide	M/1,000	88 ± 2.3 (3)	very little
Na-arsenite	M/10,000	71 ± 0.7 (3)	very little
Na-fluoroacetate	M/1,000	20 ± 2.5 (4)	good
Na-malonate	M/100	38 ± 8.2 (4)	good

* In this and the following table, the figure following the ± sign is the standard error of the mean, the figure in brackets indicates the number of determinations.
The uninhibited controls (11 determinations) consumed in 1 hour per 100 million 40.5 ± 5.2 mm^3 O_2. Their motility was excellent at the end of the run.

culture trypanosomes (literature in von Brand, 1952; von Brand, Weinbach, and Tobie, 1955), although some differences occurred. For instance, the culture form of *T. gambiense* seems to be nearly insensitive to fluoroacetate.

The respiratory quotient was 0.90. A similar, or slightly higher, figure is often reported also in work with the culture forms of other species.

Anaerobically, no gas evolution could be detected. Indeed the greater part of the relatively small amount of bound CO_2 contained in the medium disappeared during the 2-hour period of anaerobic incubation. This may indicate carbon dioxide fixation, an observation in accord with the data of Ryley (1956) on culture forms of *T. cruzi*.

Table II is a summary of our chemical data. The average rate of oxygen con-

* Fisher Company, Silver Spring, Maryland.

TABLE II.—*Metabolic endproducts and carbon balance of the culture form of* Trypanosoma congolense.

	Long term culture experiments		Short term experiments			
			Aerobic		Anaerobic	
	µg per 1 ml medium	C in percent of glucose C	mm^3 or µg per 100 million per hour	C in percent of glucose C	µg per 100 million per hour	C in percent of glucose C
Oxygen consumed	——*	——	38.3 ± 8.2 (15)	——	0*	——
RQ	——	——	0.90 ± 0.13 (7)	——	——	——
Glucose consumed	421.8 ± 34.5 (22)	100	198.7 ± 42.8 (15)	100	77.3 ± 8.2 (13)	100
Pyruvate produced	112.8 ± 19.3 (11)	25.7	75.9 ± 16.1 (7)	39.2	26.2 ± 3.3 (3)	34.5
Lactate produced	35.5 ± 9.2 (8)	7.9	19.0 ± 9.4 (6)	9.6	Trace?	0
Acetate produced	144.2 ± 15.3 (11)	32.2	46.4 ± 5.6 (3)	23.3	32.6 ± 6.0 (3)	41.9
Succinate produced	30.7 ± 4.3 (7)	6.9	10.0 ± 2.8 (2)	5.1	22.0 ± 3.0 (2)	28.6
Glycerol produced	0**	0	2.7 ± 0.3 (3)	1.3	5.2 ± 1.2 (4)	6.4
Ethylalcohol	0 (8)					
Sum of carbon percentages of metabolites		72.7		78.5		111.4
Oxygen required for complete oxidation of unaccounted sugar			31.9			

*—— denotes in this and the following table not done or not applicable. 0 denotes not consumed or not produced.
** Instead of an increase, a small decrease in glycerol values was found in 6 experiments.

sumption coincided closely with that shown in Table I which was derived from completely independent experiments. Table II also shows the resulting carbon balance. It is evident that, aerobically, keto acids and acetic acid were the main metabolic endproducts. Smaller amounts of succinic and lactic acids were also excreted. Only minor quantitative differences between the short term experiments with concentrated flagellates and the long term culture experiments were found in respect to these acids. A more profound difference may have occurred in the case of glycerol. During short term experiments a small, but consistent increase was noted, while in the long term experiments an equally consistent small decrease was observed. We are merely recording this observation without trying to interpret it because we are fully aware of the fact that the glycerol method employed is not entirely specific and, in the presence of carbohydrates, has limited sensitivity.

The aerobic carbon balance indicates that most of the carbon atoms of the metabolized glucose appear in the organic endproducts. The oxygen consumed is in excess of that required to oxidize completely the unaccounted-for carbon atoms of the sugar which disappeared. In view of the additional fact that the respiratory quotient was 0.90 instead of 1.0, it is probable that some other substance besides glucose was partly or completely oxidized.

Under anaerobic conditions the flagellates consumed appreciably less glucose than under aerobic ones. In general, aerobic invertebrates show the opposite relation (literature in von Brand, 1946), i.e. anaerobically they metabolize considerably more carbohydrate than aerobically in order to compensate at least partially for the differential in energy release between aerobic and anaerobic sugar catabolism. In animals like the present ones which live aerobically largely by fermentative processes, this point is evidently of minor importance. The fact that *T. congolense* culture forms consume only relatively small amounts of glucose anaerobically may indicate that they are not well adapted to complete lack of oxygen. This assumption is substantiated by the observation that the flagellates, although alive at the end of the

2-hour anaerobic incubation period, showed considerable clumping. This did not happen when they were incubated under air.

The anaerobic metabolic endproducts were, on the whole, similar qualitatively to those produced aerobically with the exception that anaerobically no respiratory carbon dioxide could be demonstrated and that at most traces of lactic acid were produced. It should be mentioned in this connection that the medium contained some lactic acid initially. Aerobically but not anaerobically a definite increase over the initial amount was demonstrable. It is, however, uncertain whether a true difference is involved; it could be that owing to the relatively small amount of sugar consumed anaerobically, the lactate increase here was so small as to be undetected by the method. It is evident that acetate and especially succinate anaerobically accounted for a higher percentage of the carbon atoms of the metabolized sugar than aerobically.

The carbon atoms of the anaerobic metabolic endproducts were in excess of those contained in the consumed sugar. This anomaly may be due to various causes. In describing the methods it was pointed out that due to the limited material it was not possible to determine, besides the sugar consumption, all the metabolites in a single experiment, but only one or two. This introduced the biological variation between various experiments as a source of error in addition to the analytical errors. Other possible causes of the discrepancy are: The possible origin, either totally or partially, of one or more of the metabolites from non-sugar sources, or the excretion of some reducing substance which would mask the sugar consumption, a possibility always existing when the sugar is determined by reducing methods. It should be mentioned in this connection that the glucose values have been corrected for the reducing properties of the pyruvic acid produced. It has been mentioned earlier that anaerobically some bound carbon dioxide disappeared, the carbon of which would be expected to appear in the endproducts. This amount, was however, too small to change the balance materially.

Table III shows a comparison of the carbon balances of all culture trypanosomes

TABLE III.—*Carbon balances of culture forms of various Trypanosomidae.**

	Respiratory CO	Pyruvate	Lactate	Acetate	Succinate	Glycerol	Ethylalcohol
			Aerobic				
Strigomonas oncopelti	17.4	14.2	0.6	1.7	14.6	16.0	30.2
Trypanosoma cruzi	47.8	0	0	20.7	6.7	0	0
Trypanosoma congolense	20.0	39.2	9.6	23.3	5.1	1.3	—
Trypanosoma gambiense	—	13.3	8.5	27.8	22.4	—	0
			Anaerobic				
Strigomonas oncopelti	7.7	10.5	1.0	3.1	28.8	7.9	19.3
Trypanosoma cruzi	0	0	0	20.3	32.9	0	0
Trypanosoma congolense	0	34.5	0	41.9	28.6	6.4	—
Trypanosoma gambiense	—			—	—	—	—

* The data for *Strigomonas oncopelti* and *Trypanosoma cruzi* are calculated from Ryley (1956), those for *Trypanosoma gambiense* from von Brand, Weinbach, and Tobie, (1955), those for *Trypanosoma congolense* are those discussed in the present paper. The figures are percentages of the carbon of the glucose consumed.

that have been studied so far. It is evident that both quantitative and qualitative differences exist. It would be very desirable to study more forms to determine whether or not true different metabolic patterns exist that would characterize groups of culture flagellates (corresponding to the insect-parasitizing stages), as they do in the case of the blood stream forms.

SUMMARY

1. The oxygen consumption of the culture form of *Trypanosoma congolense* is sensitive to cyanide and sulfhydryl inhibitors. It is inhibited but slightly by Krebs cycle inhibitors.

2. The RQ is 0.9. Anaerobically, no respiratory carbon dioxide is produced.

3. The major aerobic organic metabolic endproducts are pyruvate and acetate. Smaller amounts of lactate, succinate, and glycerol are excreted also.

4. Under anaerobic conditions, the main endproducts are pyruvate, acetate, and succinate. Small amounts of glycerol are produced also; lactic acid is produced at most in traces, if at all.

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A NEW FAMILY AND GENUS OF CYCLOPOID COPEPODS PARASITIC ON A HOLOTHURIAN

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During an investigation of the copepod parasites of invertebrates at Nossi-Bé, Madagascar, many specimens of the small siphonostome copepod described below were collected from the body surface of *Stichopus chloronotus* Brandt, a holothurian frequently found in that region. The copepods were recovered from the sediment obtained after washing the sea cucumbers in 5% alcohol in sea water for several minutes.

The field work was made possible by a fellowship awarded the senior author by the John Simon Guggenheim Memorial Foundation. Subsequent laboratory study has been aided by a grant from the National Science Foundation.

Nanaspidae n. fam.

Minute parasitic copepods, body oval, flattened dorsoventrally. An articulation between the head shield (formed by the fusion of the head and the segment bearing the maxillipeds) and the thoracic shield (formed by the fusion of the segments bearing legs 1–4). Genital segment and abdomen very small. First antenna showing sexual dimorphism. Second antenna without an exopodite and with a minute, weakly developed, terminal claw. No siphon, the mouth being located in an oval raised area. Mandible reduced to a minute, slender spine. First maxilla having an inner seta and a setiferous branch, and with an isolated seta near its base. Second maxilla with a well-developed claw. Maxilliped without a distinct claw.

Legs 1 and 2 biramous, legs 3 and 4 uniramous, lacking the endopodite. Leg 5 absent. Leg 6 a projecting lobe.

Caudal ramus minute. Two small egg sacs, each with usually 2 eggs. Spermatophores elongated and slender.

Nanaspis n. gen.

Female: Body minute, oval, flattened dorsoventrally. A head shield, comprising more than half of the body, articulating with a fused thoracic shield broadly indented posteriorly. Segment bearing the maxillipeds fused with the head. Segment between that of leg 4 and the genital segment weakly developd and not included in the thoracic shield. Abdomen minute, 1-segmented. First antenna 8-jointed. Second antenna 4-jointed, without an exopodite, ending in a small, weakly formed, hairy claw. Mouth located in a slightly raised oval area formed by the fusion of the labrum and the labium. Mandible represented only by a minute, slender spine. First maxilla with an inner seta and an outer setiferous branch, and with an isolated seta near its base. Second maxilla 2-jointed, the second podomere a well-developed claw. Maxilliped 5-jointed without a distinct claw.

Legs 1 and 2 biramous, leg 1 with both rami 1-jointed, leg 2 with the exopodite 3-jointed and the endopodite 2-jointed. Legs 3 and 4 lacking the endopodite, leg 3 three-jointed, leg 4 two-jointed. Leg 5 absent. Leg 6 a projecting setiferous lobe.

Caudal ramus minute. Two oval egg sacs, each usually with 2 eggs, sometimes one.

Male: Body minute, oval, flattened dorsoventrally, with the anterior border of the head shield flattened rather than rounded. First antenna prehensile and 6-jointed. Spermatophores elongated and slender. Other parts in general like those of the female.

Parasitic on the body surface of Holothurioidea.

Type species: Nanaspis tonsa n. sp.

(The generic name, derived from νᾶνος, a dwarf, and ἀσπίς, a shield, alludes to the form of the body.)

Received for publication August 22, 1958.

Nanaspis tonsa n. sp.
(All measurements in microns)

Specimens studied: 500 females and 250 males from 3 *Stichopus chloronotus* Brandt at Pointe Lokobe, Nossi-Bé, Madagascar, June 13, 1955. Holotype female, allotype, and 160 paratypes (100 females and 60 males) deposited in the Institut de Recherche Scientifique de Madagascar at Tananarive, and the same number of paratypes in the Muséum National d'Histoire Naturelle at Paris and in the United States National Museum at Washington. The remaining paratypes in the authors' collection.

Female: Body much flattened and widened, oval in contour (Fig. 1). Length, not including the setae on the caudal rami, 430 (384–456), width 318 (300–336), based on 10 specimens. Head segments and the segment bearing the maxillipeds fused, forming a broad dorsal shield with somewhat attenuated posterior corners. Head appendages concealed in dorsal view by the shield. A conspicuous articulation separating the segment bearing the maxillipeds and that of the first pair of legs. Segments bearing legs 1–4 fused, forming a rounded thoracic region whose shape completes the oval contour of the body. This region broadly indented posteriorly, where the genital segment and the abdomen are visible. Length of the head shield in relation to the length of the fused thoracic region about 6:4.

Legs 1–4 arising from the anterior half of the thoracic region and hidden in dorsal view. Segment located between the fused segments of legs 1–4 and the genital segment and which in other copepods bears the fifth legs, membranous, 30 long, often difficult to see in whole mounts, and lacking appendages (Fig. 2). Genital segment 89 × 44, narrowing posteriorly, showing ventrally 2 transverse rows of spines on its anterior half (Fig. 2). With 2 strongly sclerotized, posterolateral, setiferous lobes (Fig. 3), probably representing the sixth pair of legs, connected by a delicate ventral ridge. Abdomen 1-segmented, 20 long, deeply indented anally, with the ventral surface having rows of setae as in Figure 2.

Egg sac attachments dorsolateral on the midregion of the genital segment. Both egg sacs about 158 × 82, containing usually 2 eggs (Fig. 4), occasionally a single egg.

Rostrum not developed but perhaps represented by a slight prominence between the bases of the first antennae (Fig. 13). First antenna (Fig. 5) with 8 podomeres, their lengths 18, 15, 5, 3, 4, 4, 5, and 14 respectively. Last podomere armed with 2 aesthetasks about 60 long and 7 setae arranged as in the figure. Second antenna (Fig. 6) composed of 4 podomeres, the first with a semicircle of spinules, the second about half as long as the first and unarmed, the third as long as the first and bearing a row of broad spines along one margin and a row of delicate spines along part of its distal border, and the last in the form of a short, hairy claw (Fig. 7) about 16 long.

Mouth located in an oval raised area (Fig. 13) about 60 × 42, probably formed by the fusion of the labrum and the labium. Mandible represented only by a minute, slender spine 20 long arising on the side of the raised oval area (Figs. 8 and 13). First maxilla (Fig. 9) an elongated lobe having a plumose inner seta 65 long and an outer cylindrical branch 14 long bearing 3 naked setae 4, 54, and 48 long respectively. An isolated plumose seta 65 long located behind the base of the second antenna, not far from the first maxilla. Second maxilla (Fig. 10) 2-jointed, the first podomere large and unarmed, the second a well-sclerified, recurved claw 78 long. Maxilliped (Fig. 11) 5-jointed, the first podomere short with a few small spines, the second elongated and bearing a row of spinules along its expanded anterior edge, the third short and triangular with a single distal seta, the fourth elongated with a row of spinules and a distal seta on its posterior edge, and the fifth terminating in a hairy, brush-like process (Fig. 12). Articulation between the fourth and fifth podomeres weak. Oral area as in Figure 13. Edge of the head shield showing a few delicate hairs.

Legs 1 and 2 biramous, legs 3 and 4 uniramous, the endopodites being absent. Spine and setal formula of legs 1–4 as follows:

	leg 1		leg 2		leg 3		leg 4	
	exp	end	exp	end	exp	end	exp	end
1st podomere	3	1	1:0	0:1	1:0		1:0	
2nd podomere			1:1	4	1:1		5	
3rd podomere			6		5			

First leg (Fig. 14) with both rami 1-jointed, about 23 × 6. Basipodite with an outer seta and several long, slender inner setae. Exopodite with 3 long terminal setae and 3 minute setae. Endopodite with a single long terminal seta and numerous small setae on its surface. All setae of the first leg naked. Second leg (Fig. 15) with a 3-jointed exopodite and a 2-jointed endopodite. Coxopodite short and unarmed. Basipodite elongated, much longer than the coxopodite, and armed with 2 small setae on its outer distal corner. Exopodite with the last podomere having 3 outer spines and 3 terminal plumose setae. Endopodite with the first podomere having an inner, distal, plumose seta and the second podomere having a slender, short, naked, outer spine

and 3 plumose setae. Third leg (Fig. 16) 3-jointed with a spine-like projection near the outer spine on the first and second podomeres and with 2 outer spines and 3 plumose setae on the last podomere. Endopodite absent. Fourth leg (Fig. 17) 2-jointed, the basipodite extremely long and slender. First podomere with an outer spine, the second with 4 spines and an inner plumose seta.

Leg 5 absent. Leg 6 as in Figure 3. Caudal ramus (Fig. 18) minute, 11 × 8, with 5 slender setae and a long stout seta 96 in length, all naked, arranged as in the figure.

Color in life in transmitted light translucent, eye red, egg sacs opaque to slightly brown.

Male: Body oval (Figs. 19 and 24), similar in form to the female but with the anterior border of the head shield flattened or even slightly concave, instead of rounded. Length, not including the setae on the caudal rami, 349 (336–360), width 277 (264–288), based on 10 specimens. Tips of the first antennae visible in dorsal view. Remaining appendages and the abdomen concealed in dorsal view, the abdomen being usually curved ventrally in alcoholic specimens. Genital segment (Fig. 20) 64 × 58, its ventral surface showing 4 transverse rows of small spinules and a posterior interrupted row of slightly longer spinules. Two posterolateral lobes representing the sixth legs, each bearing 4 setae (Fig. 20), one of them longer and stouter than the others and having a few lateral hairs. Abdomen 1-segmented, about 24 long, indented anally, with the 2 parts divergent and armed ventrally with rows of spinules as in Figure 20.

Rostrum not developed. First antenna (Fig. 22) apparently 6-jointed, the first podomere with a single seta, the second of a similar size and with numerous setae on its anterior surface, the third and fourth compressed and each armed with several setae, the fifth podomere greatly swollen and displaced posteriorly (Fig. 23) and having on its anterior edge a well-sclerified process with a single seta and a broad lobe armed distally with minute spines, and the sixth podomere elongated, well-sclerotized, suggesting a claw in form, with its concave surface opposing the spinose lobe of the fifth podomere. The 2 aesthetasks and the 2 long setae on the posterior surface of the sixth podomere indicating that this podomere is homologous to the eighth podomere of the first antenna of the female. Other setae on the sixth podomere as in the figure. Tip of this podomere with a small, clear lamella.

Second antenna and mouth parts as in the female and arranged as in Figure 24. Legs 1–4 as in the female. Leg 5 absent. Leg 6 as in Figure 21. Caudal ramus as in the female.

Spermatophores long and slender, 48 × 8 (see Fig. 24).

Color in life as in the female.

(The specific name, from *tonsus,* smooth, stripped of branches, alludes to the reduced condition of legs 1–5.)

The new family Nanaspidae appears to be related to the 3 siphonostome families which also possess prehensile second antennae, namely, the Cancerillidae Sars 1915, the Micropontiidae Gooding 1957, and the Stellicomitidae Humes and Cressey 1958. In all these families the siphon is reduced or absent.

There are, however, fundamental differences between *Nanaspis* and the genera of these 3 related families, differences which in our opinion justify the creation of the new family. In the Cancerillidae (represented by *Cancerilla* Dalyell 1851, *Parartotrogus* T. Scott 1893, and *Cancerillopsis* Stephensen 1933), mostly parasites of Ophiuroidea, the thorax has free segments, the second antenna has a well-formed claw, the last podomere of the maxilliped is attenuated in the form of a sharply pointed claw, and leg 4 is rudimentary or absent.

In the Micropontiidae (represented only by *Micropontius* Gooding 1957), parasitic on Echinoidea, the thorax has free segments, the first antenna is 14-jointed in the female and 13-jointed in the male, the second antenna has a long claw, the last podomere of the maxilliped is an attenuated claw, and leg 5 is present. Legs 3 and 4 lack the endopodite, as in *Nanaspis.*

In the Stellicomitidae (represented by *Stellicomes* Humes and Cressey 1958 and *Onychopygus* Humes and Cressey 1958), parasites of Asteroidea, the body is swollen dorsally and the segmentation is not evident, the first antenna is 3- or 4-jointed, legs 1–4 are biramous, and leg 5 is present. The maxilliped is similar to that of *Nanaspis,* except that it ends in a bifid tip instead of a hairy process.

Stephopontius typicus Thompson and A. Scott 1903 deserves special mention here. This copepod, found "in the general washings from dredged Invertebrates" in Ceylon, was placed by Thompson and Scott in the Asterocheridae. Its original description is not as complete as could be wished for, but certain features suggest the location of this copepod close to the four families discussed here. The sizes given in the original description of 6.7 mm for the female and 8 mm for the male are so unusually large for siphonostomes that one might be justified in suspecting typographical errors. A close relationship to *Nanaspis* is suggested by the pronounced dividing line separating the "head" from the "thorax," the single spine representing the mandible (Thompson and Scott's "maxilla," Fig. 25), the structure of the first maxilla (Thompson and Scott's "mandible and palp," Fig. 24), the biramous leg 1 with 1-jointed rami, the lack of an endopodite in leg 4, and the lack of an articulated leg 5. *Stephopontius typicus* differs from the new genus, however, in the structure of legs 2 and 3, in the shape of the body and the apparent segmentation of the thorax, in the segmentation of the male abdomen, in the lateral appendages on the "second" thoracic segment of the female, in the 6-jointed first antenna of the female and in the 2-jointed first antenna of the male, and in showing sexual dimorphism in the caudal ramus.

It is evident that *Stephopontius typicus* needs to be restudied and more fully described. We are not venturing at this time to suggest the exact systematic position of this copepod. It seems likely that, when it is rediscovered, it will prove to be a parasite of echinoderms.

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Explanation of Plates

All figures were drawn with the aid of a camera lucida. The letters following each figure refer to the scale at which the figure was drawn.

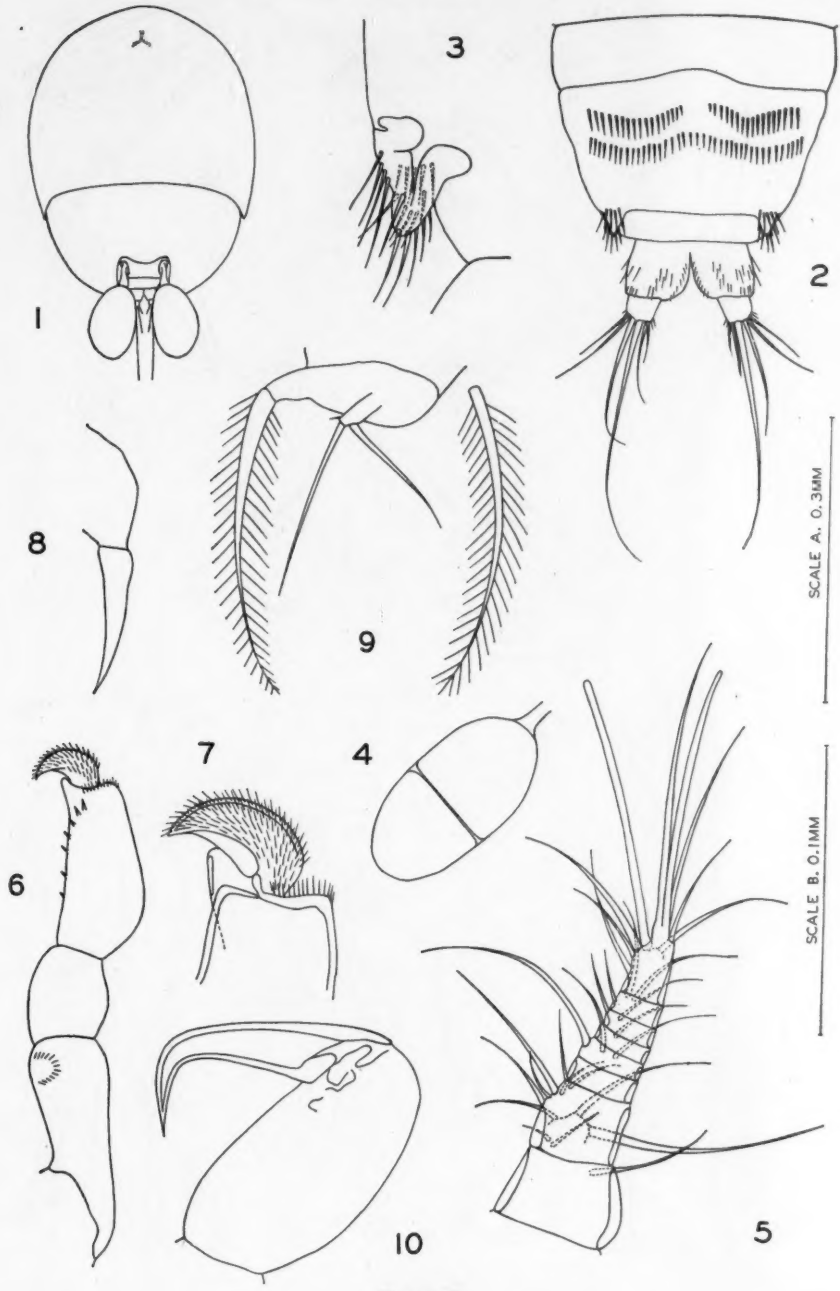

PLATE I

Nanaspis tonsa n. gen., n. sp., female

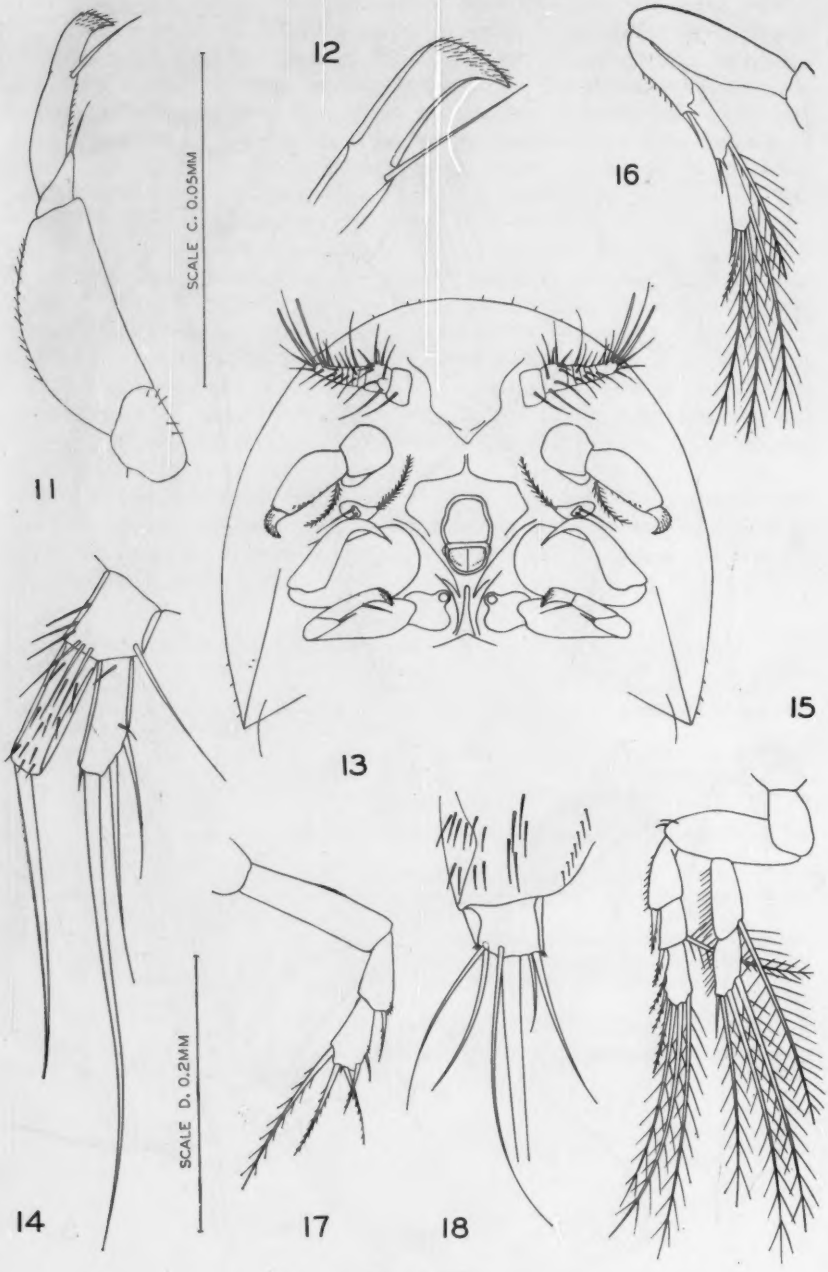

PLATE II

Nanaspis tonsa n. gen., n. sp., female (continued)

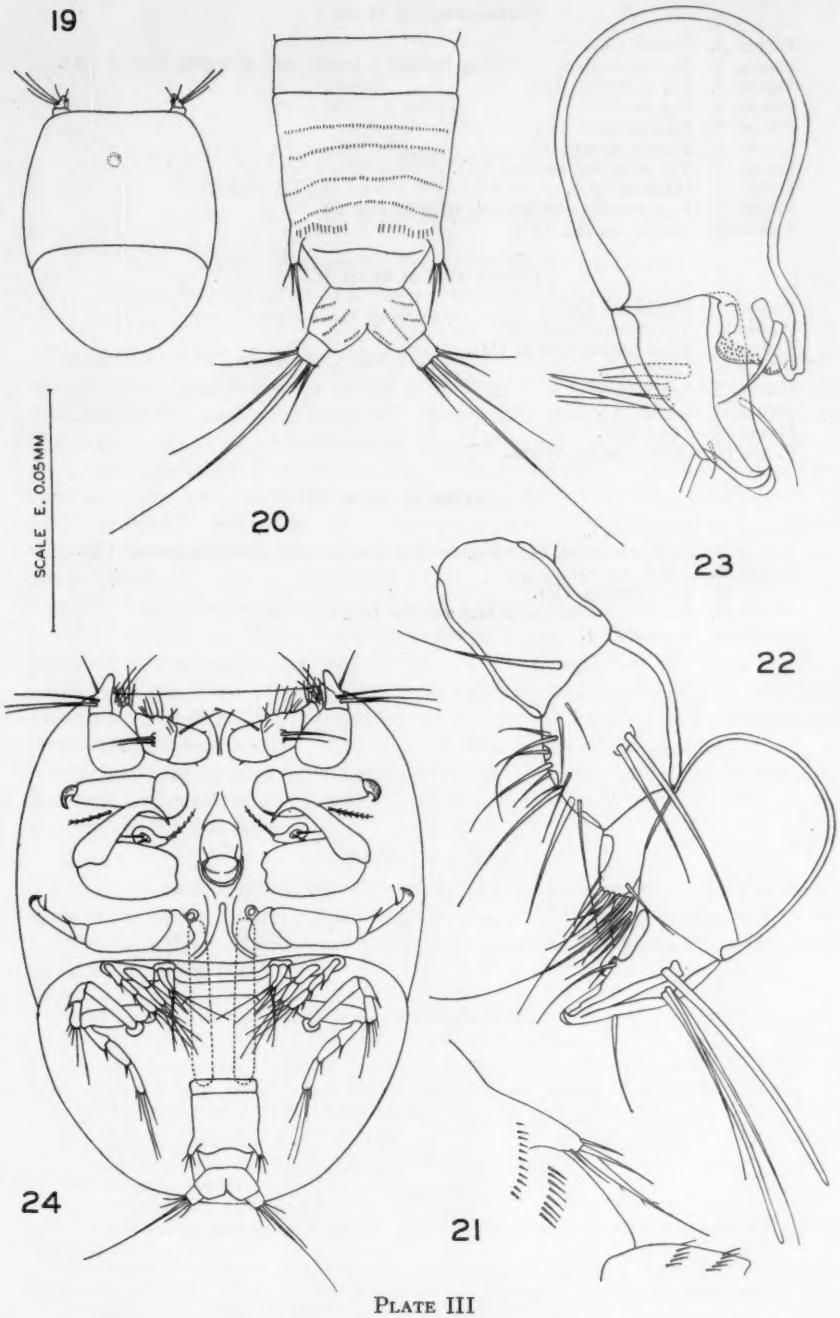

PLATE III

Nanaspis tonsa n. gen., n. sp., male

Explanation of Plate I

Figure 1. Dorsal (A).
Figure 2. Genital segment, preceding thoracic segment, and abdomen, ventral (B).
Figure 3. Leg 6, ventral (C).
Figure 4. Egg sac (D).
Figure 5. First antenna (E).
Figure 6. Second antenna (B).
Figure 7. Tip of second antenna (C).
Figure 8. Mandible (C).
Figure 9. First maxilla and isolated, adjacent seta (B).
Figure 10. Second maxilla (B).

Explanation of Plate II

Figure 11. Maxilliped (B).
Figure 12. Tip of maxilliped (C).
Figure 13. Head region, ventral (D).
Figure 14. Leg 1 (C).
Figure 15. Leg 2 (B).
Figure 16. Leg 3 (B).
Figure 17. Leg 4 (B).
Figure 18. Caudal ramus, ventral (C).

Explanation of Plate III

Figure 19. Dorsal (A).
Figure 20. Genital segment, preceding thoracic segment, and abdomen, ventral (B).
Figure 21. Leg 6, ventral (C).
Figure 22. First antenna (E).
Figure 23. Last two podomeres of first antenna (C).
Figure 24. Ventral (D).

TICKS (IXODOIDEA, IXODIDAE) PARASITIZING LOWER PRIMATES IN AFRICA, ZANZIBAR, AND MADAGASCAR.

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Garnham (1957) in showing that certain infections of African monkeys are obviously tick-borne, speculated on the possibility of a tick vector of *Hepatocystis* (= *Plasmodium*) *kochi* (Levaditi and Schoen, 1932) among these animals. African monkeys are hosts of presumably ixodid-transmitted piroplasms, *Babesia pitheci* Ross, 1905 (infecting *Cercopithecus mitis* and *C. aethiops* in various parts of the continent) and *Entopolypoides macaci* Fairbairn, 1948 (infecting *Cercopithecus* sp. in Tanganyika). Relapsing fever spirochetes presumably transmitted by argasid ticks are *Borrelia pitheci* (Théroux and Defougére, 1919) from the Soudan (French West Africa), *B. harveyi* (Garnham, 1947) of Kenya, and other unnamed forms from the Sudan, Belgian Congo, and Guinea (French West Africa).

The considerable interest that the Garnham report has aroused will obviously stimulate further research on this subject. A brief compilation of known tick-host relations of the lower primates of Africa, Zanzibar, and Madagascar is therefore indicated. The term "lower primates" as used herein includes all known primate hosts except *Homo sapiens*. The present data derive from unpublished data in collections of the present writers and of Jane B. Walker, East African Veterinary Research Organization, and from Hoogstraal (1956). More recent literature is abstracted and listed in the text.

TICK-HOST DATA

Available records are tabulated below by tick species concerned. The scientific name of the host is followed by the stage or sex of the ticks represented and by the locality where the specimens were taken. The designation "immature" includes both larval and nymphal ticks.

Amblyomma hebraeum Koch, 1844
(The South African Bont Tick)

Galago crassicaudatus zuluensis, immatures from Umfolosi Game Reserve, South Africa. *Cercopithecus aethiops* subsp., larvae from Hluhluwe Game Reserve, South Africa. *Papio ursinus subsp.*, larvae from Kanye, Bechuanaland.

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Amblyomma variegatum (Fabricius, 1794)
(The Tropical Bont Tick)

Galago senegalensis subsp., and *G. s. albipes*, immatures and adults from Karamoja, Uganda. *G. crassicaudatus agisymbanus* and *G. senegalensis zanzibaricus*, numerous nymphs from Zanzibar.

Amblyomma spp.

Cercopithecus aethiops subsp., attached to ears at Kodera, South Nyanza, Kenya (Garnham, 1957). Larvae were taken from Garnham's hosts, which were *C. aethiops johnstoni* (J. B. Walker, correspondence). *C. a. cloetei*, nymphs (?*A. pomposum sensu* Santos Dias) from Mapai, Mozambique (Santos Dias, 1954).

Haemaphysalis leachii leachii (Audouin, 1827)
(The Yellow Dog-Tick)

Galago crassicaudatus subsp., one male from Newala, Tanganyika.

Haemaphysalis lemuris Hoogstraal, 1953
(The Madagascar Lemur Haemaphysalid)

Lemur catta, adults and nymphs from Tulear District, Madagascar. *Lemur variegatus*, female from southern Madagascar. *Lepilemur ruficaudatus*, nymphs from Belkily, southeastern Madagascar. *Propithecus v. verreauxi*, female from Ambohimahavelona, Tulear, Madagascar. These records are from Hoogstraal (1953). *H. lemuris* and *H. hylobatis* Schulze, a parasite of the gibbon, *Hylobates syndactylus*, of southern Sumatra, are the only haemaphysalids and among the few arthropods to have evolved as host-specific ectoparasites of primates (see also *Ixodes schillingsi* group below).

Haemaphysalis parmata Neumann, 1905
(The West African Antelope Haemaphysalid)

Cercopithecus (=*Lasiopyga*) *c. cephus*, nymphs from Djoum, French Cameroons.

Hyalomma truncatum Koch, 1844
(The African Hyalomma)

Papio ursinus subsp., larvae from Kanye, Bechuanaland.

Hyalomma sp.

Papio ursinus subsp., larvae from Potchefstroom, South Africa.

Ixodes cavipalpus Nuttall and Warburton, 1908
(The Angolan Russet Tick)

Papio c. cynocephalus (=*Cynocephalus babouin*), males and females from Kanshanshi, Northern Rhodesia.

Ixodes rasus Neumann, 1899
(The Central African Russet Tick)

Colobus b. badius, female from N'dzida, Ivory Coast. *Cercocebus albigena* subsp., female from Okongena, Lubutu, Belgian Congo. Both records are from Arthur and Burrow (1957); previous records of this tick from primates should be disregarded owing to taxonomic revision of the tick species involved.

Ixodes lemuris Arthur, 1958
(The Madagascar Lemur Russet Tick)

Lemur macaco rufus, female from Ankafana Forest, East Betsileo, Madagascar (Arthur, 1958).

This tick is a member of the *I. schillingsi* group (see below).

Ixodes schillingsi Neumann, 1911
(The East African Colobus Russet Tick)

Colobus polykomos dodingae, female from Lotti Forest, Equatoria, Sudan. *C. polykomos matschiei*, females from Njoro, Rift Valley Province, Kenya. *C. polykomos caudatus*, males and nymphs from Mulala, Mt. Meru, Tanganyika, and numerous specimens of both sexes and nymphs from Kibonoto, Mt. Kilimanjaro, and other (unstated) localities in Tanganyika. *Galago crassicaudatus agisymbanus*, numerous immatures from Zanzibar and Pemba Islands (see paragraph below). *Colobus* sp., females from Nairobi, Cederwood, and Nyeri, Kenya. Host not stated, male and females from Gorongoza Province, Mozambique.

The numerous larvae and nymphs from greater galagos on Zanzibar and Pemba [reported by Arthur (1958) without reference to hosts; host determinations were obtained by authors in correspondence with collector, Dr. W. H. R. Lumsden] are of special interest owing to the absence of records of this otherwise *Colobus*-specific parasite from Zanzibar *Colobus* populations. Additional collecting will probably reveal that *Ixodes schillingsi* does parasitize Zanzibar *Colobus* monkeys. The presence of these ticks on insular galagos suggests that they may have evolved on galagos and later adapted to certain monkeys.

Ixodes schillingsi is known definitely from *Colobus* monkeys, the Zanzibar greater galago, one female from man at Njoro (near Taveta), Kenya (Lumsden, 1955), and one female from a crested rat, *Lophiomys ibeanus* subsp., Nanyuki, North Nyeri, Kenya (Hoogstraal). The crested rat was an immature captive caged near various species of monkeys from which it probably adventitiously acquired the parasite. West African records from other monkeys have now been relegated to *Ixodes rageaui* by Arthur (1958). For biology of this tick, see Hoogstraal (1956, pp. 559–560). These ticks are common in small numbers on the eyelids but up to 12 are sometimes found in the axillae of their hosts.

The *Ixodes schillingsi* group includes the species *schillingsi* of East African *Colobus* monkeys, *lemuris* of Malagasy lemurs, and *rageaui* of West African *Cercopithecus* monkeys.

Only 2 other ixodids host-specific on primates have been recorded, these are *Haemaphysalis lemuris* of Malagasy lemurs and *H. hylobatis* of Sumatran gibbons.

Ixodes sp.

Perodicticus potto, Ixodes sp. (stage or sex not stated), French Cameroons (Morel and Mouchet, in press).

Rhipicephalus appendiculatus Neumann, 1901
(The Brown Ear-tick)

Galago crassicaudatus zuluensis, larvae from Umfolosi Game Reserve, South Africa. *Cercopithecus aethiops* subspp., immatures from Hluhluwe Game Reserve, South Africa, and from Inkberry, East London, South Africa; and (Garnham, 1957) in ears of hosts at Kodera, South Nyanza, Kenya. The hosts reported by Garnham are *C. aethiops johnstoni;* nymphs from them molted to adult *R. appendiculatus.* Both larvae and nymphs were taken on the hosts (J. B. Walker, correspondence). *Papio ursinus* subsp., numerous immatures from Potchefstroom, South Africa.

Rhipicephalus e. evertsi Neumann, 1897
(The Red Tick)

Papio ursinus subsp., larvae from Kanye, Bechuanaland.

Rhipicephalus pravus Dönitz, 1910
(The East African Convex-eyed Brown Tick)

Papio cynocephalus, adults from Mchichira, Newala District, Tanganyika.

Rhipicephalus s. sanguineus (Latreille, 1806)
(The Kennel Tick)

Galago crassicaudatus agisymbanus and *G. senegalensis zanzibaricus,* immatures from Zanzibar. *Galago senegalensis* subsp., (stage not stated) from Bakel, Senegal, French West Africa (Villiers, 1955). *G. s. senegalensis,* male from Imurok, Torit, Equatoria, Sudan. *Cercopithecus aethiops nesiotes,* larvae from Zanzibar. *Cercopithecus aethiops* subsp., numerous adults from captive individuals, Torit, Equatoria, Sudan. *C. aethiops* subsp., "Many engorged (stage and sex not stated) on ears" at Kodera, South Nyanza, Kenya (Garnham, 1957). *"Monkey",* females from Doruma, Belgian Congo. *Papio doguera heuglini,* adults from Kenisa, Bahr El Ghazal, Sudan.

Rhipicephalus s. simus Koch, 1844
(The Glossy Tick)

Galago crassicaudatus agisymbanus, adults from Zanzibar. *Papio doguera ibeanus,* adults from Gede, Kenya (Lumsden, 1955). *P. doguera heuglini,* numerous members of several large baboon families in Bahr El Ghazal Province were free of ticks but old male hobos wandering alone were infested by several to 200 adult *R. s. simus,* chiefly in the hosts' axillae. *Colobus polykomos matschiei,* adults from Njoro, Rift Valley Province, Kenya.

HOST-TICK DATA

Available records for ticks infesting lower primates are listed below under the

individual host concerned. The stage or sex (L = larva, N = nymph, M = male, F = female) is followed by the tick species and by the collecting locality. The supraspecific designations of primates are after Simpson (1945). Specific and common names are from a variety of sources; unfortunately no two of the many authorities on primates come even close to agreement on the taxonomy of forms in the area under consideration.

PRIMATES

PROSIMII

LEMURIFORMES, LEMURIDAE

Lemur catta Linnaeus. Maki, or ring-tailed lemur.
N,M,F, *Haemaphysalis lemuris*, Tulear District, Madagascar.
L. variegatus Kerr. Ruffed lemur.
F, *Haemaphysalis lemuris*, southern Madagascar.
L. macaco rufus Audebert (=*L. rufifrons* Bennett). Black lemur.
F, *Ixodes lemuris*, Ankafana Forest, East Betsileo, Madagascar.
Lepilemur ruficaudatus A. Grandidier. Black-tailed lemur.
N, *Haemaphysalis lemuris*, Bekily, southeastern Madagascar.

LEMURIFORMES, INDRIDAE

Propithecus v. verreauxi A. Grandidier, Sifaka.
F, *Haemaphysalis lemuris*, Ambohimahavelona, Tulear, Madagascar.

LORISIFORMES, LORISIDAE

Perodicticus potto subsp. Potto.
?, *Ixodes* sp. French Cameroons.
Galago crassicaudatus zuluensis Elliot. Zulu greater galago.
L,N, *Amblyomma hebraeum*, Umfolosi Game Reserve, South Africa.
L, *Rhipicephalus appendiculatus*, Umfolosi Game Reserve, S. Africa.
G. crassicaudatus agisymbanus (Coquerel). Zanzibar greater galago.
N, *Amblyomma variegatum*, Zanzibar.
L,N, *Ixodes schillingsi*, Zanzibar and Pemba.
L,N, *Rhipicephalus s. sanguineus*, Zanzibar.
M,F, *Rhipicephalus s. simus*, Zanzibar.
G. crassicaudatus subsp.
M, *Haemaphysalis l. leachii*, Newala, Tanganyika.
G. s. senegalensis E. Geoffroy. Senegal bushbaby.
M, *Rhipicephalus s. sanguineus*, Imurok, Torit, Equatoria, Sudan.
G. senegalensis albipes Dollman. Kavirondo bushbaby.
L,N,F,M, *Amblyomma variegatum*, Karamoja, Uganda.
G. senegalensis zanzibaricus Matschie. Zanzibar bushbaby.
N, *Amblyomma variegatum*, Zanzibar.
L,N, *Rhipicephalus s. sanguineus*, Zanzibar.
G. senegalensis subsp.
L,N,F,M, *Amblyomma variegatum*, Karamoja, Uganda.
?, *Rhipicephalus s. sanguineus*, Bakel, Senegal, F.W.A.

ANTHROPOIDEA

CERCOPITHECIDAE

Cercocebus albigena subsp. Mangabey.
F, *Ixodes rasus*, Okongena, Lubutu, Belgian Congo.
Papio ursinus subsp. Chacma baboon.
L, *Amblyomma hebraeum*, Kanye, Bechuanaland.
L, *Hyalomma truncatum*, Kanye, Bechuanaland.
L, *Hyalomma* sp., Potchefstroom, South Africa.
L,N, *Rhipicephalus appendiculatus*, Potchefstroom, South Africa.
L, *Rhipicephalus e. evertsi*, Kanye, Bechuanaland.
P. c. cynocephalus (Linn.) (=*Cynocephalus babouin* Desmarest). Yellow baboon.
M,F, *Ixodes cavipalpus*, Kanshanshi, Northern Rhodesia.
M,F, *Rhipicephalus pravus*, Mchichira, Newala, Tanganyika.

P. doguera heuglini Matschie. Sudan anubis baboon.
M,F, *Rhipicephalus s. sanguineus*, Kenisa, Bahr El Ghazal, Sudan.
M,F, *Rhipicephalus s. simus*, various localities, Bahr El Ghazal, Sudan.
P. doguera ibeanus Thomas. Kenya anubis baboon.
M,F, *Rhipicephalus s. simus*, Gede, Kenya.
Cercopithecus aethiops cloetei Roberts. Transvaal guenon.
N, *Amblyomma* sp., Mapai, Mozambique.
C. aethiops nesiotes Schwarz. Pemba green monkey.
L, *Rhipicephalus s. sanguineus*, Zanzibar.
C. aethiops johnstoni Pocock. Kilimanjaro Green Monkey.
L,N, *Amblyomma* sp. Kodera, South Nyanza, Kenya.
L,N, *Rhipicephalus appendiculatus*, Kodera, South Nyanza, Kenya.
?,?, *Rhipicephalus s. sanguineus*, Kodera, South Nyanza, Kenya.
C. aethiops subsp.
L, *Amblyomma hebraeum*, Hluhluwe Game Reserve, South Africa.
M,F, *Rhipicephalus appendiculatus*, Hluhluwe Game Reserve and Inkberry, East London, S. Afr.
M,F, *Rhipicephalus s. sanguineus*, Torit, Equatoria, Sudan.
C. (=*Lasiopyga*) *cephus cephus* (Linnaeus). Moustached guenon.
N, *Haemaphysalis parmata*, Djoum, French Cameroons.
M,F, *Ixodes rageaui*, Yaounde, French Cameroons.
"MONKEY"
F, *Rhipicephalus s. sanguineus*, Doruma, Belgian Congo.
Colobus b. badius (Kerr). Red colobus.
F, *Ixodes rasus*, N'dzida, Ivory Coast.
C. badius kirkii Gray. Zanzibar colobus.
L,N, *Ixodes schillingsi*, Zanzibar.
C. polykomos dodingae Matschie. Sudan colobus.
F, *Ixodes schillingsi*, Lotti Forest, Equatoria, Sudan.
C. polykomos matschiei Neumann. Kenya colobus.
F, *Ixodes schillingsi* Njoro, Rift Valley Province, Kenya.
C. polykomos caudatus Thomas. Tanganyika colobus.
L,N,M,F, *Ixodes schillingsi*, Mt. Meru and Mt. Kilimanjaro, Tanganyika.
Colobus sp.
F, *Ixodes schillingsi*, Nairobi, Cederwood, and Nyeri, Kenya.

DISCUSSION

Although spirochetes of African monkeys, *Borrelia pitheci*, *B. harveyi*, and *B.* spp., are presumed to be transmitted by argasid ticks, no records of *Argas* or *Ornithodoros* ticks biting lower primates in nature are available. None of the ixodid species mentioned above as parasitizing primates in Africa and islands adjacent to it have yet been incriminated in the epidemiology of several assuredly tick-borne diseases. A rich field of research obviously awaits students with facilities to study these relationships.

The Zanzibar and Karamoja records noted above are from material obtained by Drs. W. H. R. Lumsden and A. Haddow. The Potchefstroom and Bechuanaland data were mostly obtained by Dr. F. Zumpt.

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We are grateful to Professor P. C. C. Garnham, Dr. W. H. R. Lumsden, Mr. Glen M. Kohls, and Miss Jane B. Walker for offering information and suggestions.

SUMMARY

Certain pathogens of lower primates in Africa, Zanzibar, and Madagascar belong to genera that are consistently tick-borne, e.g. *Babesia pitheci* Ross, 1905, *Entopolypoides macaci* Fairbairn, 1948, *Borrelia pitheci* (Théroux and Defougére, 1919),

B. harveyi (Garnham, 1947), and *B.* spp. In order to assist further research on the vectors and epidemiology of these organisms, we present here records of ticks attacking pottos, galagoes, bushbabies, lemurs, monkeys, and baboons on continental Africa, Zanzibar, and Madagascar; these concern *Amblyomma hebraeum* Koch, *A. variegatum* (Fabricius), *Haemaphysalis l. leachii* (Audouin), *H. lemuris* Hoogstraal, *H. parmata* Neumann, *Hyalomma truncatum* Koch, *Ixodes cavipalpus* Nuttall and Warburton, *I. rasus* Neumann, *I. lemuris* Arthur, *I. rageaui* Arthur, *I. schillingsi* Neumann, *Rhipicephalus appendiculatus* Neumann, *R. e. evertsi* Neumann, *R. pravus* Dönitz, *R. s. sanguineus* (Latreille), and *R. s. simus* Koch.

The tick species host-specific on lower primates throughout the world are *Haemaphysalis lemuris, H. hylobatis* Schulze (of Sumatran gibbons), *Ixodes lemuris, I. rageaui* and *I. schillingsi.*

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STERNOSTOMA KELLOGGI, A NEW SPECIES OF NASAL MITE FROM THE CATBIRD (ACARINA: RHINONYSSIDAE)

KERWIN E. HYLAND, JR.* AND DAVID T. CLARK**

Recently Furman (1957) has revised the genus *Sternostoma* Berlese and Trouessart, 1889, to include 9 species from a variety of bird hosts. Of the 9 species considered in the revision 5 are known from North America.

A series of specimens collected from the nasal cavity of the catbird (*Dumetella carolinensis*, family Mimidae) in southern Michigan differs from all others and is here described as new. All measurements are in microns.

Sternostoma kelloggi, n. sp.

Female (Figs. 3-8)

Idiosoma—706 long, 389 wide.

Dorsum—Propodosomal plate 271 long, 235 wide, nearly pentagonal in outline with anterior border forming an antero-median projection, lateral borders slightly convex, corners rounded, with a minute punctate pattern throughout and with a median closely set, vacuolate area. Ten pairs of alveoli, each alveolus bearing a minute seta. Opisthosomal plate 172 long, 134 wide, rectangular, lateral margins slightly concave, slightly narrower posteriorly, corners rounded, minute punctate pattern with scattered vacuolate areas, 6 pairs of alveoli, 2 pairs of which bear minute setae. Stigmata dorsally located, circular, 25 in diameter, without peritremes, but with a small tube extending posteriorly from the stigma as described by Strandtmann (1951) for *S. boydi*. Unarmored dorsal integument weak, with fine striations, and without setae or accessory plates.

Venter—Sternal plate rectangular, with irregular borders, 134 long, 109 wide, lateral margins slightly convex; surface pattern of minute punctations laterally and with transverse striae concentrated medially; 3 pairs of alveoli, each alveolus with a minute seta. Genito-ventral plate triangular in shape with rounded apex directed posteriorly; basal margin slightly convex, 140 long, 112 wide; surface with mosaic background; center of plate vacuolated; no setae present. Anal plate circular, 31 in diameter, weakly sclerotized without cellular design, alveoli, or setae. One pair of setae lie lateral to plate. Unarmored integument weak, with striations and with 2 pairs of minute and closely set setae between anal and genito-ventral plates.

Legs—Leg I: length to tip of claw 456, length of claw 64; coxa 140 wide. Leg IV: length to tip of claw 392, claw 56; coxa 106 wide. Legs I, II, and III with 2 alveoli on each coxa, each alveolus with a minute seta. Coxa IV without alveoli or setae but with vacuolate areas. Dorsum of coxa I with a row of 7 or more short projections forming a ctenidium-like structure on the anterior distal area. Tarsus I bears apically 4 relatively large attenuate setae, a sensory rod about ½ the length of the attenuate setae, and 4 minute setae. Tarsi II, III, and IV each with 1 attenuate subapical seta, and ventrally with 2 transverse rows of 3 setae each, the distal row composed of 3 papillate setae some being acute and/or spiraled and the proximal row composed of 2 papillate setae with a short, blunt, loaf-shaped seta between; 2 narrow generalized setae situated dorsally and 1 ventrally. All setae without cross striations. All remaining leg segments with 1 or more minute setae per segment. Claw and ambulacrum on leg I greatly reduced; claws of legs II, III, and IV stout, hooked and similar in size and shape. Caruncles of legs II to IV tripartate and similar.

Gnathosoma—Ventral in position, with approximately ½ the length visible from dorsal aspect. Palps with 4 movable segments, each segment at least as broad as long; tarsus with 2 larger and

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about 7 minute apical setae; other movable segments with 1 or more minute setae. Chelicerae 112 long, 22 wide at base, attenuated; chelae small, 6 in length. Hypostome ventral with 2 pairs of minute setae.

Male (Figs. 1–2)

Idiosoma—456 long, 294 wide.

Dorsum—Propodosomal plate 216 long, 207 wide, same general shape as in female holotype and with same ornamentation, but lateral limits very poorly defined. Opisthosomal plate 134 long, 92 wide, same shape and ornamentation as in female, but lateral margins ragged and ill-defined; 6 pairs of alveoli present, 2 pairs of which bear minute setae. Stigmata dorsally located, oval, without peritremes. Unarmored dorsal integument weak, without evidence of setae but with some faint striations.

Venter—Sternal plate elongate with convex borders, 378 long and 179 wide. Posterior third constricted forming a posterior lobe 123 wide and containing a central vacuolate pattern. Male genital opening surrounded posteriorly by anterior margin of plate, opening 39 in diameter; lateral and posterior to the opening, plate is more heavily sclerotized and has strong closely set punctations; central area of sternal plate with a mixture of weak punctations and striae; 3 pairs of alveoli, each alveolus with a minute seta. Genito-ventral plate 101 long, 62 wide, oval, weak and without ornamentation. Anal plate oval, 67 long. A pair of minute setae lateral to plate.

Legs—Leg I: length to tip of claw 319, length of claw, 22; coxa 95 wide. Leg IV: length to tip of claw 283, claw 28; coxa 90 wide. Coxae I, II, and III with alveoli and setae, and coxa I with ctenidial structure same as in female. Tarsal setation similar to female.

Gnathosoma—Ventral in position with approximately ½ its length visible in dorsal view. Palps similar to female; chelicerae chelate, stouter than female, 66 long, chelae 25 long.

Material—Seven females and 1 male collected from the nasal cavity of a single specimen of the catbird, *Dumetella carolinensis*, taken at 42nd Street and Brook Lodge Road, Kalamazoo County, Michigan, on 3 July 1958 by K. E. Hyland, D. T. Clark, and Hedwig E. Geiger constitute the type material. Holotype (female), allotype, and 1 female paratype have been deposited in the United States National Museum, Washington, D. C. (U. S. N. M. No. 2506). Two paratypes each will be deposited in the Entomological Museum of Michigan State University, and the collection of the senior author. The remaining paratype will be deposited in the University of California collection.

DISCUSSION

Sternostoma kelloggi appears to have greater affinities with *S. tracheacolum* Lawrence, 1948, *S. hutsoni* Furman, 1957, and *S. cryptorhynchum* Berlese and Trouessart, 1889, than with other species of the genus. On the basis of dorsal and ventral plates it is closer to *S. hutsoni*, but may be separated from it by the presence of attenuate setae on the distal ends of tarsi II, III, and IV, and by the presence of setae in the alveoli of the sternal plate. *S. kelloggi* can be separated from *S. tracheacolum* by the presence of a greater number of alveoli and setae on the propodosomal and opisthosomal shields, by the presence of alveoli with setae on coxae I, II, and III, and a greater number of attenuate setae on tarsus I. It may be separated from *S. cryptorhynchum* on the basis that the 6 specialized setae on tarsi II, III and IV are not spatulate as in *S. cryptorhynchum*, and that *S. kelloggi* possesses a greater number of setae on the propodosomal shield and has a differently shaped genito-ventral plate.

In addition to the 9 species of *Sternostoma* considered by Furman in his revision, Fain (1956a, 1956b) has described 11 new species from Africa. It is difficult to ascertain the position of these since there are no drawings and the descriptions are rather brief. The authors agree with Furman in considering Fain's forms distinct.

S. kelloggi is named in honor of the late W. K. Kellogg whose Michigan estate is now the Kellogg Gull Lake Biological Station.

SUMMARY

A new rhinonyssid mite, *Sternostoma kelloggi*, is described from male and female specimens taken from the nasal cavity of the catbird, *Dumetella carolinensis*, collected in Kalamazoo County, Michigan.

EXPLANATION OF PLATE
Sternostoma kelloggi, n. sp.

Male
FIGURE 1. Genito-ventral plate.
FIGURE 2. Chelicera.
Female
FIGURE 3. Dorsal view. P—propodosomal plate; O—opisthosomal plate.
FIGURE 4. Tarsus I.
FIGURE 5. Tarsus II, ventral view.
FIGURE 6. Gnathosoma, ventral view.
FIGURE 7. Genital plate.
FIGURE 8. Ventral view.

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RESEARCH NOTE

AUTOLYSIS AS AN AID TO CYSTICERCOID COUNTING IN EXPERIMENTAL *HYMENOLEPIS NANA INFECTIONS*

Much of the experimental work with the dwarf mouse tapeworm, *Hymenolepis nana*, employs a count of infected mouse villi containing fully developed cysticercoids. The technique of Hunninen (1935, J. Parasitol. **21**: 124–125) involves removal of the intestine from infected mice, and slitting, refrigerating, and washing flattened two-inch sections prior to examination of the gut strips under a dissecting microscope. A modification of this procedure, which permits rapid handling of large amounts of material, and a less obstructed view of individual villi, has been used successfully in this laboratory. The small intestines of infected mice are removed at the appropriate time (90–100 hours after feeding of eggs) and are placed unopened in tap or distilled water in petri dishes and refrigerated for 48 hours. Bacterial and autolytic breakdown are permitted to occur during this time, slowed and controlled by the duration of refrigeration. At the end of this period partially sloughed mesenteries and outer lining membranes can be quickly removed, and the intestines split and carefully washed clear of digested matter, chiefly the mucoid mass lining the gut wall. This autolytic process loosens or destroys the mucus and other debris which fills the crypts between villi, masking the cysticercoids, and making counts tedious and often unreliable.

The original technique of Hunninen removes this material, but it is often necessary to wash each section repeatedly and to remove adherent debris under a dissecting microscope before an accurate cysticercoid count is possible. The present technique both clears the debris and permits rapid removal of outer fascia and tissues as well, so that a single washing clears and exposes each villus with minimum handling. Proper control of exposure time and refrigeration temperature assures control of the autodigestive process so that neither the villi nor the cysticercoids are affected. By the use of this procedure it is also considerably easier to locate newly emerged worms in the partially digested wash material, while those adhering to the gut wall can be located and counted *in situ*, as scraping of the mucosa is unnecessary, and the majority of worms remain attached and are unaffected by the autolysis. An example of the examination made possible by this procedure is the fact that one can identify villi previously infected, but no longer containing cysticercoids, and discern the cysticercoid linings left behind inside the villi. This permits differentiation between successive infections or allows a greater laxity in the precise time of removal of the gut in the case of single infections.—DONALD HEYNEMAN AND BARBARA A. SINDELL, *Department of Zoology, University of California, Los Angeles, California.*

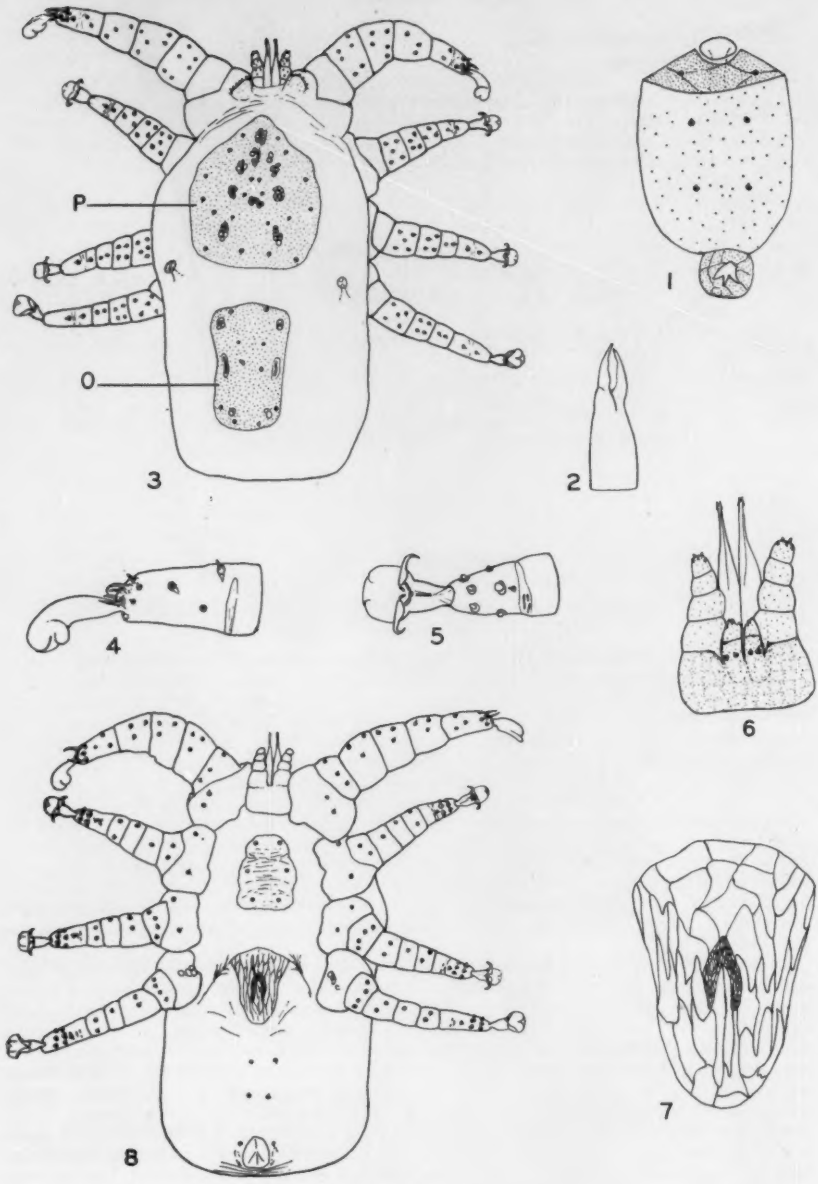

PLATE I

BIOLOGICAL OBSERVATIONS ON CERTAIN TURKISH *HAEMAPHYSALIS* TICKS (IXODOIDEA, IXODIDAE).

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INTRODUCTION

These data comprise the only recorded detailed field observations, outside the U.S.S.R., on immature-stage hosts of 4 economically important haemaphysalid species. The material was collected by the writer and M. N. Kaiser during NAMRU-3 expeditions to western Turkey (Istanbul, Sapanca, Izmit, Abant, and Lake Emir near Ankara) in August and the first half of September, 1954, and to eastern Turkey (Tatvan, Van, Edremit, Norsinc, Urfa, Kara Kopru, and Iskenderun) in July and the first half of August, 1954.

Collections of Turkish *Haemaphysalis* ticks in the British Museum (Natural History) and Rocky Mountain Laboratory were examined through the courtesy of Mr. E. Browning and Mr. G. M. Kohls, respectively. Their data are included here, and the source is indicated by the symbols BM(NH) or RML.

PARASITE DATA

Haemaphysalis erinacei taurica Pospelova-Shtrom, 1939

3 males, ex 3 *Erinaceus europaeus concolor*, Van, Van, 18 July.
23 males, 11 females, 5NN, ex 3 *Vulpes v. ?anatolica*, Van, Van, 15 July.
8 males, 11 females, ex *Vulpes v. ?anatolica*, Van, Van, 16 July.
3 males, ex 2 *Vulpes v. ?anatolica*, Kara Kopru, Urfa, 6 July.
1L, ex *Allactaga w. williamsi*, Edremit, Van, 15 July.

Through the kindness of Professor Maria Pospelova-Shtrom we have received sufficiently long series of Asiatic specimens in all stages to enable us to confirm her opinion that the species *H. erinacei* (= *H. numidiana*) consists of 3 subspecies: *H. e. erinacei* Pavesi, 1884, in North Africa, *H. erinacei taurica* Pospelova-Shtrom, 1939, in the Near East including western U.S.S.R., and *H. erinacei turanica* Pospelova-Shtrom, 1939, in central Asia.

Although the subspecies *taurica* and *turanica* show more adaptability to non-insectivore hosts than the subspecies *erinacei*, they still retain a limited range of host predilection and seldom parasitize domestic animals. A high degree of host specificity characterizes all phylogenetically advanced haemaphysalids. This group of species, all with palpi widely salient basolaterally in each stage, seldom attacks domestic animals except dogs.

Kurtpinar (1954) lists "*H. numidiana*" from Ankara (Middle Anatolian High Steppe Biological Province). Our records are from the Syrian and Armenian Highland Biological Provinces. This species is undoubtedly more widely distrib-

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The opinions and assertions contained herein are the private ones of the author and are not to be construed as official.

uted in Turkey than present records indicate but additional search, especially of foxes and hedgehogs, will be necessary to reveal its range and incidence.

H. erinacei is considered to be a possible reservoir or vector of plague among small mammals (Pomerantzev, 1950).

Haemaphysalis otophila Schulze, 1918

1L,	ex *Erinaceus europaeus concolor,* Tatvan, Bitlis, 28 July.
1N,	ex *Allactaga w. williamsi,* near Norsinc, Bitlis, 27 July.
4NN,	ex 3 *Allactaga w. williamsi,* near Norsinc, Bitlis, 25 July.
3NN,	ex 2 *Allactaga w. williamsi,* Edremit, Van, 19 July.
1N,	ex *Meriones blackleri intraponticus,* Lake Emir, Ankara, 18 August.
1N,	ex *Meriones blackleri intraponticus,* Lake Emir, Ankara, 19 August.
1N,	ex *Meriones blackleri intraponticus,* Lake Emir, Ankara, 21 August.
8LL, 17NN,	ex *Spalax leucodon,* Kamer, Tatvan, Bitlis, 30 July.
10NN,	ex *Spalax leucodon,* 30 miles west of Bolu, Bolu, 11 August.
4NN,	ex *Cricetulus migratorius cinerascens,* Bolu, Bolu, 13 August.
1N,	ex *Microtus g. guentheri,* Lake Emir, Ankara, 20 August.
3NN,	ex *Microtus g. guentheri,* Lake Emir, Ankara, 21 August.
1N,	ex *Mus musculus musculus,* Lake Emir, Ankara, 22 August.
1N,	ex *Apodemus sylvaticus tauricus,* Tatvan, Bitlis, 24 July.
2 males,	ex domestic sheep, Istanbul abattoir, 29 August.
9 males, 6 females,	ex domestic cattle, Inanli, Kirklareei, 16 Nov. 1947 (RML)
1 female,	from beach, Zonguldak, 5 November 1948 (Schulze collection, RML).

Owing to the usual cold season feeding period of adult *H. otophila,* very few individuals of this stage were taken during our expeditions to Turkey, and the 2 females on sheep in late August probably represent about the earliest autumn feeders. The data for immature-stage hosts noted above widely extend the known range of these stages on rodents.

This species was recorded from Afyon, Ankara, Bolu, Bursa, Canakkale, Denizli, Elazig, Erzurum, Eskisehir, Gumuskane, Malatya, Samsun, Sivas, Tekirdag, Tokat, Urfa, and Van (Kurtpinar, 1954). It is therefore, widely distributed in Turkey. The density of populations in different biological provinces is unknown.

Haemaphysalis punctata Canestrini and Fanzago, 1878

1N,	ex *Erinaceus europaeus concolor,* Sapanca, Kocaeli, 4 August.
1N,	ex *Erinaceus europaeus concolor,* Sapanca, Kocaeli, 5 August.
3 females,	ex domestic goat, Smyrna (Izmir) (Nuttall lot 2553A), BM(NH).
1 female,	ex domestic sheep from Kars, Istanbul abattoir, 3 September.
9NN,	ex domestic sheep, Sapanca, Kocaeli, 5 August.
1L,	ex *Pica pica,* Kamer, Tatvan, Bitlis, 27 July.
64LL, 6NN,	ex partridge, 10 miles south of Abant, Bolu, 11 August.
8LL,	ex *Turdus merula,* Abant, Bolu, 10 August.
1N,	ex *Corvus monedula soemmeringi,* Sapanca, Kocaeli, 4 August.

Adults of *H. punctata* usually feed in autumn and spring though in some areas also in winter. The record of nymphs from sheep is relatively uncommon. The number of larvae and nymphs from 4 birds, as compared to the rare nymphs from the numerous hedgehogs and small rodents that we examined, suggests that birds are the preferred hosts of these stages as reported by numerous Soviet workers.

In eastern Turkey we took only a single larva (Bitlis). Localities where *H. punctata* has been taken in Turkey are Afyon, Ankara, Balikesir, Bilecik, Bolu, Canakkale, Cankiri, Coruh, Denizli, Elazig, Gaziantep, Hakkari, Kars, Malatya, Manisa, Mardin, Samsun, Tekirdag, and Urfa (Kurtpinar, 1954). This species, therefore, is widely if unevenly distributed in Turkey.

Protozoal diseases of domestic animals and rickettsiae of human disease are

transmitted by this tick, which also causes human and animal paralysis and has been experimentally associated with the transmission of *Brucella melitensis* (Pomerantzev, 1950).

Haemaphysalis sulcata Canestrini and Fanzago, 1878

1N,	ex *Erinaceus europaeus concolor,* Kara Kopru, Urfa, 7 August.
1N,	ex *Vulpes v. ?anatolica,* Kara Kopru, Urfa, 6 August.
1L,	ex *Allactaga w. williamsi,* Van, Van, 15 July.
1L,	ex *Allactaga w. williamsi,* Edremit, Van, 19 July.
1N,	ex *Pica pica,* Kamer, Tatvan, Bitlis, 27 July.
1N,	ex *Apathya cappadocica,* Urfa, Urfa, 4½ miles north of, 6 August.
4NN,	ex *Agama stellio,* Kara Kopru, Urfa, 6 August.
5NN,	ex *Ophisops elegans,* Urfa, Urfa, 3 miles north of, 3 August.
1 female,	ex domestic cattle, Uzak, Helmar *legit* (Schulze collection, identified by him as ?*H. angorensis*—RML).
1 male, 3 females,	ex domestic goat, Saraykay, Denizli (RML No. 25260).

Adults of *H. sulcata* feed during the cooler months of the year. Larvae and nymphs prefer reptiles as hosts, but sometimes attack birds and rarely feed on small mammals. The data above confirm these host predilections in Turkey.

Our material was secured only in eastern Turkey, in the Armenian Highland and Syrian Biological Provinces. Kurtpinar (1954) lists *H. puncata* only from Ankara, Bolu, Malatya, Silvas, Tekirdag, and Van. Wintertime collecting and careful examination of lizards will probably reveal the more extensive distribution of *H. sulcata* in Turkey. This species, according to Pomerantzev (1950), may transmit brucellosis and may cause paralysis in animals.

HOST DATA

Erinaceus europaeus concolor Martin, 1838. Near Eastern Hedgehog.
H. erinacei taurica, 3 males, Van. *H. otophila,* 1L, Tatvan. *H. punctata,* 2NN, Sapanca. *H. sulcata,* 1N, Kara Kopru.

Four of the 31 hedgehogs examined in Turkey were lightly infested by 1 of 4 haemaphysalid species. All hosts were taken in gardens, orchards, pastures, or weedy fields near buildings. Hedgehogs in the Sapanca area were also infested by nymphs and females of *Ixodes ricinus* (Linnaeus).

Vulpes vulpes ?anatolica Thomas, 1920. The Anatolian Red Fox (= ?*V. v. kurdistanica* Satunin, 1906).
H. erinacei taurica, 3 males, Kara Kopru; 31 males, 22 females, 5NN, Van. *H. sulcata,* 1N, Kara Kopru.

The 58 specimens of *H. erinacei taurica* at Van were taken from a mother and 2 immature foxes living in a rocky cave above Lake Van. Two other foxes roaming on nearby slopes were free of haemaphysalids. The 2 hosts of the 4 ticks from Kara Kopru were running on a rocky hillside in a semidesert area. No foxes were examined in western Turkey.

Allactaga w. williamsi Thomas, 1897. Williams' Kurdistan Jerboa.
H. erinacei taurica, 1L, Edremit. *H. otophila,* 5NN, near Norsinc; 3NN, Edremit. *H. sulcata,* 1L, Van; 1L, Edremit.

The rate of haemaphysalid infestation of these fascinating jerboas is fairly low, only 11 specimens having been taken from 6 of the 30 animals examined. These long-eared jerboas burrow in weedy fields above lakes or ponds, and are also infested by larvae of *Ixodes ricinus* (Linnaeus).

Meriones blackleri intraponticus Neuhäuser, 1936. Turkish Jird.
H. otophila, 3NN, Lake Emir.

We took 6 Turkish jirds from burrows in dense low weeds on a sloping steppe facing Lake Emir. They fed in nearby wheatfields or watermelon patches. Three of these animals each carried a single larval *H. otophila* (as well as *Ixodes laguri* subsp.).

Spalax leucodon Nordmann, 1840. Lesser Mole Rat.
H. otophila, 8LL, 17NN, Tatvan; 10NN, 30 mi. west of Bolu.

The host from near Bolu inhabited a tunnel in the grassy edge of a wheatfield. The Tatvan area host burrowed in a hayfield. Two others from a hayfield near Tatvan and the steppes near Lake Emir bore no haemaphysalids.

Cricetulus migratorius cinerascens Wagner, 1848. Near Eastern Migratory (or Gray) Hamster.
H. otophila, 4NN, Bolu.

The infested hamster was dug with a bulldozer from a fallow wheatfield. Six others from fields in various areas of the Anatolian steppes were free of ticks.

Apodemus sylvaticus tauricus Barrett-Hamilton, 1900. Persian Field Mouse.
H. otophila, 1N, Tatvan.

Approximately 75 of these mice were examined for ectoparasites. The single infested animal was trapped in a dry hillside forest of stunted oaks at 5800 feet altitude.

Microtus g. guentheri Danford and Alston, 1880. Günther's Anatolian Vole.
H. otophila, 4NN, Lake Emir.

Two of 12 voles from the grassy edges of wheatfields near Lake Emir were infested but others, taken on grassy slopes above Lake Abant, Bolu, were free of ticks.

Mus musculus musculus Linnaeus, 1758. House Mouse.
H. otophila, 1N, Lake Emir.

This mouse, the only small mammal infested by haemaphysalids that we took in a swampy or humid wooded area, was trapped in a weedy wooded swamp beside Lake Emir. Other genera of ticks were frequently found on mammals in such situations. Numerous house mice trapped in buildings were free of ticks. This swamp-inhabiting host was one of the few commensal mice found outside of buildings.

Domestic Sheep.
H. otophila, 2 males, Istanbul abattoir. *H. punctata,* 1 female, Istanbul abattoir (from Kars); 9NN, Sapanca.

Domestic Goats.
H. sulcata, 1 male, 3 females, Saraykay (RML). *H. punctata,* 3 females, Smyrna (Izmir) (BMNH).

Domestic Cattle.
H. sulcata, 1 female, Uzak (RML). *H. otophila,* 9 males, 6 females, Inanli.

Owing to the time of the year in which we worked in Turkey, very few haemaphysalids were among the thousands of ticks that we collected from domestic mammals.

Birds.
Pica pica (Raven): *H. punctata,* 1L. *H. sulcata,* 1N, both Tatvan.
Turdus merula (Blackbird): *H. punctata,* 8LL, Abant.
Corvus monedula soemmeringi (Jackdaw): *H. punctata,* 1N, Sapanca.
"Partridge": *H. punctata,* 64LL, 6NN, Abant, 10 miles south of.

We examined approximately 75 birds for ticks while in Turkey. The present evidence suggests that if we had made a greater effort to obtain ground-feeding birds we might have obtained many more haemaphysalids.

Lizards.
Apathya cappadocica: H. sulcata, 1N, Urfa.
Ophisops elegans: H. sulcata, 5NN, Urfa.
Agama stellio: H. sulcata, 4NN, Kara Kopru.

Several hundred reptiles were examined in Turkey. A few in Urfa district, where some 100 lizards were taken, were infested by haemaphysalids. These lizards were captured among rocks in semiarid plains or beside irrigated orchards.

DISCUSSION

We especially attempted to rediscover *H. angorensis* Schulze and Schlottke (1927) in Turkey. This questionably valid species was described from 2 Turkish females that were lost when the Stuttgart Museum was bombed during the war (Professor Shuz, Director; personal correspondence). I have examined large numbers of Near Eastern haemaphysalids in a fruitless effort to recognize *H. angorensis.*

Kurtpinar (1954) lists *H. inermis* subsp. from Ankara, Bilecik, Coruh, Giresun, Kastamonu, Samsun, Tekirdag, Tokat, and Zonguldak, and *H. concinna* Koch,

1844, from Coruh, Ordu, and Sinop. Assuming the Ankara record to be obviously from the abattoir, both species appear to be confined to coastal areas of the Black Sea and western lowlands near the Aegean Sea.

The biological provinces of Turkey were nicely outlined by Neuhäuser (1936) on the basis of rodent distribution. While each of the 4 haemaphysalid species that we found occur in most of these biological provinces, the density of population in each is poorly known.

We carefully searched for ectoparasites on approximately 400 small land mammals representing 30 forms. Haemaphysalids were found on 32 hosts listed above. Wild mammals entirely free of haemaphysalids were: 5 *Muscardinus avellanarius trapezius,* 2 *Pitymys subterraneus fingeri,* 6 *Clethrionomys glareolus ponticus,* 2 *Mesocricetus auratus brandti,* 4 *Arvicola terrestris hintoni,* 10 *Crocidura* spp., 12 *Mus musculus brevirostris,* 9 *Mus musculus praetextus,* 6 *Citellus citellus xanthoprymnus,* 20 *Sciurus a. anomalus,* 2 *Talpa c. caeca,* 7 *Lepus europaeus syriacus,* 2 *Ellobius lutescens,* 2 *Dryomys nitedula* subsp., 1 *Microtus* sp., 1 *Meriones* sp., 3 *Rattus* sp., and 9 *Apodemus flavicollis* subsp.

Many of these animals were trapped in swamps or humid forests, but haemaphysalid hosts were almost without exception from steppelands, fallow fields, wheatfields, hayfields, pastures, gardens, or dry stunted oak-forests.

Because its fauna includes 5 primitive species, *H. i. inermis, H. concinna, H. punctata, H. otophila,* and *H. sulcata,* Turkey is an exceptionally interesting area for study of haemaphysalid biology. Each of these species is morphologically archaic and immature stages retain varying degrees of primitive host predilection for reptiles and birds. Their adults, however, have adapted widely to domestic animals and have thus ensured the survival and spread of the species. Biological studies on host relations of these ticks are especially desirable owing to the variety of human and animal diseases with which they have been associated.

SUMMARY

The only detailed field observations of immature-stage hosts, outside of the U.S.S.R., are given for 4 of the 6 *Haemaphysalis* species occurring in Turkey. These are: *H. erinacei taurica* Pospelova-Shtrom, 1939, larvae from jerboas, and nymphs and adults from hedgehogs and foxes; *H. otophila* Schulze, 1918, larvae and nymphs from hedgehogs, jerboas, jirds, mole rats, hamsters, voles, and field mice; *H. punctata* Canestrini and Fanzago, 1878, larvae and nymphs from hedgehogs, sheep, a raven, a partridge, a jackdaw, and a blackbird; and *H. sulcata* Canestrini and Fanzago, 1878, larvae and nymphs from hedgehogs, foxes, jerboas, a raven, and three kinds of lizards. Hosts were found in steppelands, pastures, fallow fields, wheatfields, hayfields, gardens, and a dry stunted oak forest, never in humid forests and only once in a swamp. Two additional species are localized in Turkey, *H. i. inermis* and *H. concinna.* All of these haemaphysalids have been associated with a wide variety of human and animal diseases.

ADDENDUM

After this paper was submitted for publication, Mr. Glen M. Kohls provided the following additional data on Turkish haemaphysalids in the Rocky Mountain Laboratory collections. These are listed below, and together with the records in the body of this report, comprise probably most of the information on these ticks

from Turkey available from American and European collections. All specimens listed below are adults; the month of collection is unknown.

H. concinna: from cattle and domestic buffalo, Of, Trabzon; Merkez, Sinop; Ordu, Uniye; and Coruh, Hopa.

H. inermis subsp.: from cattle, sheep, goats, and domestic buffalo; Merkez, Sinop; M. Kamal pasa, Bursa; and Hopa, Coruh.

H. otophila: from cattle, sheep, and goats: Bitlis; Kelkit, Gumushane; Ankara; Kirklareli, Inanli; Henisehir, Bursa; Agri, Aleskir; Tavas, Denizli; and Acipayam, Denizli.

H. punctata: from cattle and sheep, Askale, Erzurum; Corum, Iskilip; Kirklareli, Inanli; Niksar, Tokat; Acipayam, Denizli; Boyabat, Sinop; Gerze, Sinop; Merkez, Sinop; and Ankara.

H. sulcata: from cattle, goats, sheep and camel, Emet, Kutahya; Tavas, Denizli; Saraykoy, Denizli; Acipayam, Denizli; Boyabat, Sinop; and Gerze, Sinop.

The Rocky Mountain Laboratory material was furnished by Dr. S. Bilal Golem, Dr. H. Kurtpinar, and Dr. H. Sukru Otyun.

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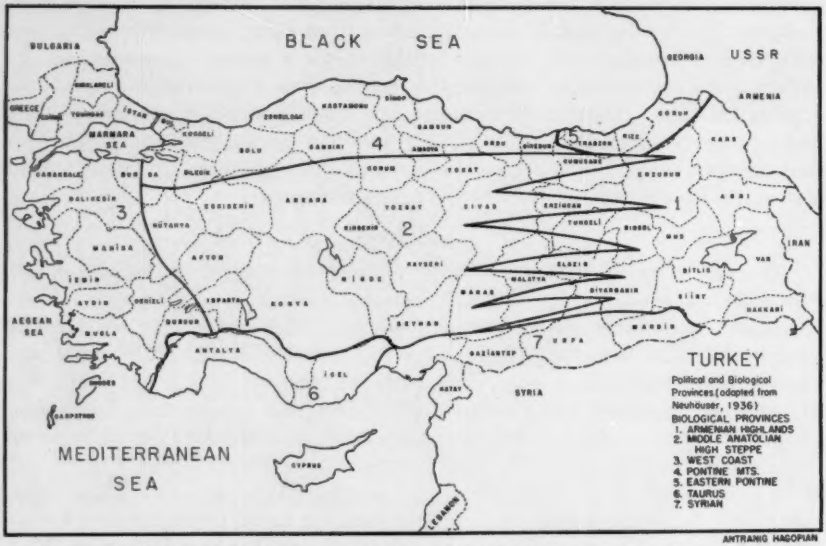

FIGURE 1. The biological provinces of Turkey, and Provinces noted in the text; modified from Neuhäuser (1936).

THE NATURE OF PARASITISM OF THE OPOSSUM BY FLEAS IN SOUTHWESTERN GEORGIA

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The common opossum, *Didelphis marsupialis*, is unusual among North American mammals in having few parasites of its own. In the United States and South America, it appears to be the normal host of but one mite, *Neoichoronyssus wernecki*, which is related to species that infest rodents (Morlan and Strandtmann, 1949), and in South America of a short-winged beetle, *Amblyopinus henseli*, also one of a group that apparently originated as parasites of rodents (Seevers, 1955). Otherwise, the opossum's ectoparasites consist of a large number of general feeders, such as triatomine bugs (Usinger, 1944) and ticks, and certain mites and fleas which, having lost or deserted their normal hosts, temporarily adopt the opossum.

Extensive observations of host relation and seasonal abundance of ectoparasites from southwestern Georgia were reported by Morlan (1952) during investigations of murine typhus fever. The present paper presents additional, detailed information on fleas from opossums based on Morlan's field data.

PROCEDURES

The opossums were taken by live-trapping in Decatur and Thomas counties from October 1945 to January 1949; in Grady County from April 1946 through April 1949; and in Brooks County between August 1946 and April 1947.

Beginning in April 1946, practically all buildings on rural premises in Thomas and Brooks Counties were treated with DDT dust. These treatments were very effective in reducing the number of Oriental rat fleas, *Xenopsylla cheopis*, on rats and cases of murine typhus fever in man (Hill and Morlan, 1948). Grady and Decatur counties were the check areas; no dusting of rural premises with DDT for control of rat fleas was done in these counties.

One hundred and twenty-one of the opossums were from buildings on premises occupied by humans in counties in which no DDT dusting was done for control of rat fleas, and 95 were from occupied premises in counties in which practically all buildings had been treated. They were taken by month from January through December as follows: From buildings in which no control had been done: 2, 4, 12, 6, 17, 4, 12, 12, 22, 20, 5 and 5. From treated buildings: 9, 9, 2, 3, 1, 4, 13, 12, 20, 12, 3 and 7.

Because infestations of opossums taken from buildings on vacated premises were similar to those of opossums caught in fields and woods, records from these were combined. None of the vacated premises had been treated with DDT dust. One hundred and thirty-three opossums were taken from fields, woods, and buildings on vacated premises by month as follows: 4, 2, 6, 21, 19, 18, 17, 16, 19, 6, 2, and 3.

Only data from animals that were taken alive were used in this study. The animals were placed in fine-mesh cloth bags, tagged with collection data, and brought to the laboratory where they were examined (Morlan, 1952). The percentage of opossums that were infested and the average number of fleas per infested opossum were calculated for each month. The annual percentage and mean are based on these monthly averages.

OBSERVATIONS

House-mouse and Human Fleas: House-mouse fleas (*Leptopsylla segnis*) were uncommon on opossums; only one was observed on each of 3 opossums, all from

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occupied buildings in February, September, and December. The human flea, identified as *Pulex irritans*, was found on only 1 opossum. Smit (1958) points out that those *Pulex* infesting non-ungulate hosts may be *P. simulans*, often mistaken for *P. irritans*.

Rabbit Fleas: The spiny rabbit flea (*Odontopsyllus multispinosus*) was observed on only 3 opossums (in February and March). One flea was found on each.

The eastern rabbit flea (*Cediopsylla simplex*) was present on 7 opossums during the period January–May and on 1 in August. The greatest mean, 2 per infested opossum, occurred in January and March.

Squirrel Flea: The squirrel flea (*Orchopeas howardi*) infested opossums during the period December–June. Fifteen percent of the opossums from untreated buildings on occupied premises and 7% of those from occupied and treated buildings were infested. The observed mean number of fleas was 1.4 and 0.5 per infested individual in the untreated and treated counties, respectively. Of the opossums from outdoors and vacant buildings 9% were infested. The mean number per infested opossum was 2.4. The greatest percentage of infestation occurred during January, February, and March. The greatest number on any one opossum was 7 in January.

Oriental Rat Flea: Oriental rat fleas, *Xenopsylla cheopis*, were taken from opossums from untreated buildings in March, May, August, and September and from buildings in treated counties in June, July, September, and October. They were not found during other months although they were present on domestic rats throughout the year (Morlan, 1952). Of the opossums caught in buildings on occupied premises, 7% were infested, but Oriental rat fleas were found on only 1 opossum caught off occupied premises. The greatest number on a single opossum was 3, in September.

Sticktight Flea: Eighteen percent of the opossums from untreated buildings were infested by sticktight fleas (*Echidnophaga gallinacea*) and 12% from treated buildings were infested. The mean number of fleas was 7.3 and 1.8, respectively. These fleas were observed on opossums in every month but January and March.

Only 1% of the opossums from outdoors and vacant premises were infested. The mean number per infested opossum was about 4. The greatest individual infestation was 138 fleas on a male opossum in June. The greatest percentage of infestation (75 and 42) occurred in June and July on occupied premises.

Cat Flea: The opossum harbored cat fleas, *Ctenocephalides felis*, during every month of the year. The percentage of opossums infested varied from 0 to 75 (mean 38) on untreated premises, from 0 to 62 (mean 35) on treated premises and from 0 to 44 (mean 21) in fields, woods and unoccupied premises. The highest prevalences of infestation were found in July and August and the smallest in winter. The mean numbers of fleas per infested opossum were 6.0 for untreated premises, and 3.3 for treated premises and for fields, woods and unoccupied farm premises.

The greatest number found on one individual was 135 on a male in May.

Cotton-Rat Flea: The opossum harbored cotton-rat fleas (*Polygenis gwyni*) during all months but December. The percentage of infestation was 20 for untreated premises, and 29 for DDT-treated premises. The mean number of fleas per infested opossum was 1.6 and 2.5, respectively. Thirty-five percent of the opossums from fields, woods, and buildings on vacant premises were infested. The mean number per infested opossum was 4.3. Greatest infestation found on one individual was 58 on a male in March. Highest percentage of infestation in any one month was 72 in July, out-of-doors, and next highest, 67 in August.

DISCUSSION

In many mammalian species, males have larger home ranges than females and therefore travel more extensively, presumably exposing themselves to more kinds of ectoparasites. The small number of opossums studied revealed no definite difference in infestation of the two sexes.

In many species, there is a marked tendency for individuals to follow beaten trails of one kind or another, thereby exposing them to such ectoparasites as are dropped by certain other hosts. Young animals usually travel extensively after weaning in order to find unoccupied habitats. Some species tend to follow wheel tracks or paths. However, in Northeastern Kansas, opossums were found to have little inclination to follow beaten trails, either of their own or those of other mammals (Fitch and Sandidge, 1953). They did, however, habitually follow intermittent creeks, gullies, and, to some extent, roads when these provided better foraging than did the adjoining habitat. No doubt opossums in Georgia have similar habits which must bear a relation to the ectoparasite load.

Opossums commonly use cavities in trees as nests, and hence are exposed to some extent to ectoparasites of squirrels (*Sciurus niger* and *S. carolinensis*) and raccoons (*Procyon lotor*). They tend to feed on the same kinds of foods as do raccoons, and to frequent the same kinds of habitat. Some of those examined in this study bore raccoon ticks as well as squirrel fleas. Raccoons in southwest Georgia were without raccoon fleas.

Opossums wander extensively into fields and thinly wooded areas, therefore into habitats of field-inhabiting mammalian species. They may nest in abandoned dens of skunks or other animals. In this study they frequently were found nesting in barns and sheds.

Pulex irritans or a related flea, *P. simulans*, was present on roof rats (*Rattus rattus*), striped skunks (*Mephitis mephitis*), and spotted skunks (*Spilogale putorius*), but infested more skunks (5 of 212) than other species (Morlan, 1952). Only 1 opossum of 350 was infested by this species, which may indicate either limited association of opossums and primary hosts of this flea or scarcity of *Pulex* in the study areas.

Leptopsylla segnis occurred on only 3 of the opossums and there was only 1 house-mouse flea on each. During the same period when the opossums were taken, 21% of the Norway rats (*Rattus norvegicus*), 19% of the roof rats, and 13% of the house-mice (*Mus musculus*) bore house-mouse fleas and the mean numbers per infested individual were 6.4, 5.4, and 3.3, respectively (Morlan, 1952). These data indicate that, although opossums frequently occur in habitats such as occupied farm buildings where the sustaining hosts (domestic rats and mice) are common, the house-mouse flea is relatively reluctant to attack or incapable of infesting opossums.

The Oriental rat flea was present on only 4% of all opossums trapped, although 216 of the opossums were from buildings on occupied farm premises where they had good opportunity to acquire these ectoparasites. Over 90% of these premises in Thomas County, and probably also in the other counties, were infested by rats in 1946 (Smith, 1957). Thirty-nine percent of the Norway rats, and 21% of the roof rats in the study counties were infested by Oriental rat fleas and the mean number per infested rat was 7 for Norway rats and 5 for roof rats (Morlan, 1952). The Oriental rat flea therefore showed little taste for or ability to exist on opossums.

The sticktight flea occurred on 9% of all opossums trapped. There is general agreement that they are primarily poultry fleas.

Cat fleas were present on a large percentage (35 to 38) of opossums from buildings on occupied farm premises during a period when every cat examined bore them (Morlan, 1952). Very nearly every occupied premises was inhabited by cats and dogs, their normal hosts. One might suspect that opossums were sustaining hosts if cats were not infested more heavily and if opossums from fields, woods, and vacant farmsteads did not have a lower prevalence (21%) of infestation. An investigation to determine if flea larvae can mature in opossum nests might shed light on this subject of host status.

Cotton-rat fleas were present on a large percentage (20 to 29) of the opossums from buildings on occupied premises and a larger percentage (35) of those from fields, woods, and buildings on vacant premises. The relatively small difference in infestation from the two types of habitats may be due to the fact that cotton rats (*Sigmodon hispidus*) commonly occur right up to, and occasionally temporarily in barns and sheds. Opossums wander about these habitats in search of food, thus replenishing their supply of cotton rat fleas which probably gradually desert opossums remaining long in buildings. Fifty-two percent of the cotton rats were infested (Smith and Love).

The spiny and eastern rabbit fleas were present on few opossums. Thirty-eight percent of the cottontail rabbits (*Sylvilagus floridanus*) taken at the same time the opossums were taken were infested by the spiny rabbit flea and the mean number of fleas per infested rabbit was 2.7. Thirty-nine percent of the cottontail rabbits were infested by the eastern rabbit flea and the mean per infested rabbit was 3.3 (Morlan, 1952). Opossums may acquire their rabbit flea infestations through their joint occupancy of habitats with rabbits.

The squirrel flea infested about from 1% to 15% of all opossums trapped, whereas over half of the gray squirrels (*Sciurus carolinensis*) and fox squirrels (*Sciurus niger*) were infested. Fox squirrels bore an average of 10, gray squirrels 3.2, and flying squirrels (*Glaucomys volans*) 5.0 individuals, respectively (Morlan, 1952). The opossum apparently derives its moderately high infestation from squirrels of these several species through use of the same habitats, including buildings and in some cases the same nests.

Use of DDT to control Oriental rat fleas apparently had little effect on the percentage of opossums infested by cat and cotton rat fleas.

SUMMARY AND CONCLUSIONS

A study was made in southwestern Georgia of the nature of parasitism by fleas of the common Virginia opossum, *Didelphis marsupialis*. The populations of fleas on opossums were compared to the populations on key or sustaining hosts of each species of flea.

Pulex fleas were present on only 1 of the 349 opossums examined. House-mouse fleas, which apparently are more dependent on commensal rats than mice in Georgia, were found on only 3 opossums of the 216 trapped in buildings on occupied premises. Oriental rat fleas were found on 7%; sticktight fleas on 12% of the opossums from DDT-treated buildings and on 18% of those from untreated buildings; cat fleas on 35% of those from treated buildings and 38% of opossums caught in treated buildings. Spiny and eastern rabbit fleas were present on only 3 and 7 opossums re-

spectively. Squirrel fleas infested up to 15% of the opossums trapped in buildings. Cotton rat fleas were present on 20% of the opossums in treated buildings and 29% from untreated buildings on occupied premises and on 35% of those from fields, woods, and vacated buildings.

Opossums from occupied farm premises were more frequently infested by cat fleas, sticktight fleas, and Oriental rat fleas than opossums caught in open country and vacated farmsteads. Infestations on 95 opossums from buildings on DDT-treated premises were not significantly different from infestations on 121 opossums from untreated buildings.

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RESEARCH NOTE

PRESUMED HATCHING OF TAENIID EGGS IN THE LIVERS OF RATS

Incidental to our studies of early and late immunity to larvae of *Hydatigera taeniaeformis* we attempted to inject eggs of this tapeworm directly into liver tissue, using surgical techniques. Known numbers of eggs or freed onchospheres were introduced into one of the large lobes of the liver by a syringe and 24-gauge needle, care being taken to avoid loss or spilling of the material. Subsequently, larval cysts were found in all of the operated animals. The number of cysts was small, about 1/20 the number found in control animals. Apparently embryos (freed onchospheres) from predigested eggs establish themselves more readily than those from untreated eggs; but in one case a dose of 100 untreated eggs resulted in the development of 4 cysts.

Although the number of animals used in our experiments was small (14) the results demonstrate (1) that rats can be infected by direct injection of cestode eggs into the liver and (2) that these eggs need not have been subjected to artificial digestion.

Three explanations may be tentatively offered, for these results. First, it is possible, but we think unlikely, that the experimental rats may have acquired infection by the normal (gastrointestinal) route, accidentally. We have had no such accidents among our many rats used over a 4-year period in other experiments. Second, it is possible that injected eggs happened to reach bile passages, through which entry into the intestine, with subsequent "normal" hatching, may have occurred. We cannot rule this possibility out. On the basis of control data, however, which in some experiments showed 7% infectivity in orally infected rats, an escape of 30 to 50 eggs from an injected dose of 100 would have been required to produce the cysts found. Movement of such high proportion of eggs seems unlikely, unless they had been injected directly into a large bile passage. Third, if the first two explanations may be discarded, there remains the likelihood of enzymatic action occurring in the liver, dissolving the cementing substance of the embryophore, but failing to destroy the embryo. While this idea is at present purely hypothetical, we think it may be tested by in vitro and in vivo experiment.

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RESEARCH NOTES

AN ANOMALY IN THE GENITALIA OF *DIPYLIDIUM CANINUM*

A single abnormal mature proglottid was discovered in a group of 6 complete specimens of *Dipylidium caninum* which were removed from the intestinal tract of a female mongrel dog. This dog had been impounded by the City of Tallahassee, Florida, and was obtained for use by the Department of Physiology, Florida State University.

The worms were alive when removed; they were relaxed in cold tap water for two hours and then smoothed onto a glass plate where they were fixed with aceto-formol-alcohol applied with a camel's hair brush. All the worms were stained as whole mounts with Harris' hematoxylin, cleared in terpineol and mounted in damar. Detailed examination of all 6 worms revealed only this single abnormal proglottid. The proglottid was studied as a whole mount and in frontal sections at 15 microns.

Figure 1 shows the position of the abnormal genital complex; Figure 2 shows details of

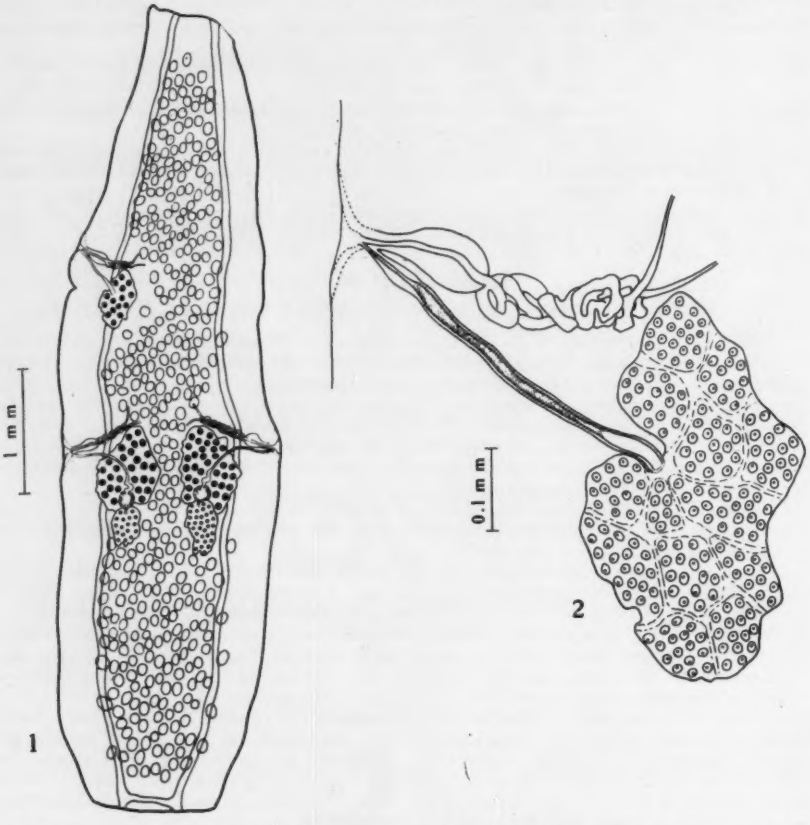


FIGURE 1. Abnormal proglottid, drawn from whole mount, with the aid of a microprojector.
FIGURE 2. Detail of abnormal genitalia, drawn from sections, with the aid of a microprojector.

the complex as seen in sections. The proglottid measured 6.42 mm long and 1.76 mm wide in the region of the normal reproductive organs.

The sections revealed clearly the abnormal anatomy of the supernumerary genitalia. The vitelline gland, oötype and Mehlis' gland were missing and the vagina ended blindly in a lateral depression on the ovary. The cellular detail in the ovary appeared normal, showing 13 large follicles containing developing egg cells. The somewhat sinuous vagina traversed a distance of 0.416 mm from the genital atrium to the ovary. The lumen of the vagina had a normal complement of cilia except at its slightly enlarged junction with the ovary. This enlargement apparently represents a seminal receptacle which is present at the proximal end of the vagina in normal *D. caninum* and which is not ciliated. The male organs in this system appeared normal in structure and 2 vasa efferentia were observed connecting testes to the sperm duct. The cirrus pouch measured 0.104 mm in length and the coiled sperm duct running from the cirrus pouch to the vasa efferentia measured 0.218 mm. The cirrus was typical and prostate cells were present around the sperm duct. There were approximately 344 testes associated with the 3 sets of genitalia, probably indicating a normal complement for each.

A comparison revealed the organs in the anomalous genitalia to be smaller than the corresponding organs in the normal complex. The normal ovary was 0.754 mm long and 0.416 mm wide at its widest point while the abnormal ovary was 0.458 mm long and 0.239 mm wide. The normal sperm duct was 0.302 mm long and the normal cirrus pouch was 0.130 mm long.

The only other similar condition in *D. caninum* known to this writer was that reported by Honigberg (1944, Tr. Am. Micr. Soc. **63**: 342–344). He described, apparently from a whole mount, a gravid proglottid which contained an extra set of reproductive organs on the left side. He indicated that all 3 genital complexes were complete and were capable of normal reproduction.

The author wishes to express graditude to Dr. Robert B. Short for advice and criticism in this study.—Rhodes B. Holliman, *Department of Biological Sciences, Florida State University, Tallahassee, Florida.*

SOME NEMATODE PARASITES OF TREE FROGS, TOADS, LIZARDS, AND LAND CRABS OF THE BERMUDA ISLANDS

During the summer of 1957, while conducting entomological and parasitological investigations in the Bermuda Islands, tree frogs, toads, lizards and land crabs were examined for external and certain internal parasites. No external parasites were recovered from any of them. The genera and species of nematodes recovered are herewith reported from the Bermuda Islands for the first time. The identifications of these nematodes were made by Mrs. M. B. Chitwood of the U.S.D.A., Agricultural Research Service, Animal Disease and Parasite Research Division.

Tree Frog—*Eleuthrodactylis johnstonei* Barbour. Eighteen of 26 specimens examined were infected with 1 or 2 of the following genera: *Cosmocerca* sp., *Aplectana* sp. or *Thelandros* sp. All were found in the ileum, rectum or cloaca. The largest number found in any given animal was 12.

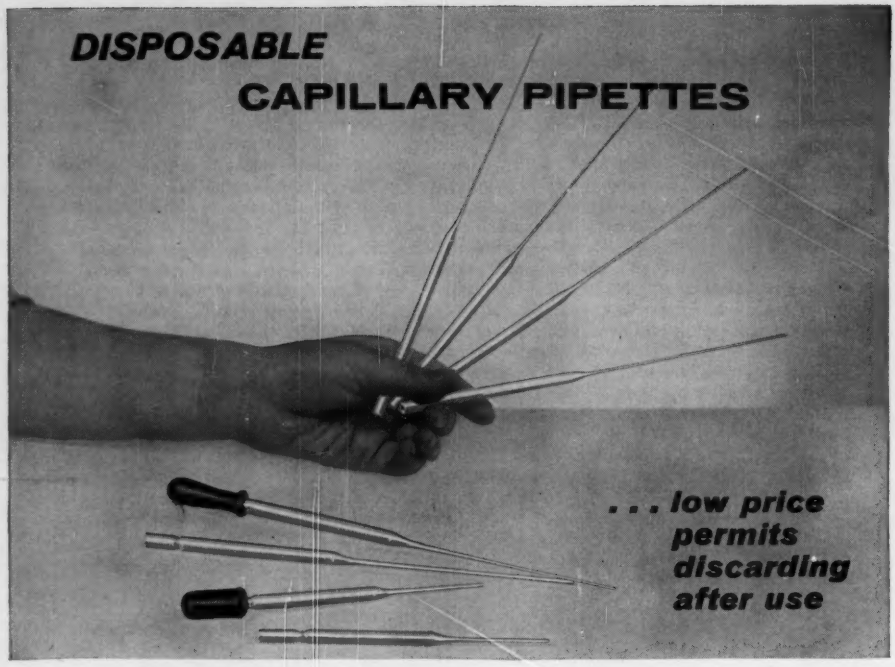
Tree Frog—*E. luteolus* Grosse. Two of 3 specimens examined contained *Pharyngodon armatus* Walton, 1933 in the ileum and rectum. The largest number recovered from a single animal was 8.

Toad—*Bufo marinus* L. Thirty-three of 40 toads examined contained hermaphroditic adults of *Rhabdias sphaerocephala* Goodey, 1924 in the lungs, 50 being the largest number recovered. Ten contained up to 130 adults of *Aplectana vellardi* Travassos, 1926 in the rectum and cloaca.

Lizard—*Anolis leachii* Dum. Three of 6 specimens examined were parasitized by up to 80 adults of *Atractis carolinea* Harwood, 1932, in the rectum and deep in the muscosa of the rectum. *A. leachii* is a previously unreported host for this nematode parasite and, therefore, represents a new host record.

Lizard—*A. grahamii* Gray. All of 23 examined were negative for nematode parasites.

Land Crab—*Gecarcinus lateralis* Freminville. All of 17 specimens examined contained up to thousands of *Tripylium carcinicolum* (Baylis, 1915) Cobb 1920, in the connective tissue, hypodermis beneath the carapace, pericardium, pericardial sacs and gills. Some also contained larval worms of the genus *Monhystra* in some of the same locations.—Roger W. Williams, *School of Public Health and Administrative Medicine, Columbia University.* Publication No. 246 from the Bermuda Biological Station. Aided by research grant E-1350 from the National Institutes of Health, Public Health Service and from the Child Frick Fund of the Bermuda Biological Station.



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